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DOPAMINE, CALCIUM CHANNELS AND THE ROLE OF CONDITIOINING IN COCAINE-INDUCED BEHAVIOURS

by

ALLAN RICHARD REIMER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

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DEDICATION

To my Family: Mother, Nina, to the memory of my Father, Jake and brother, Eric, to my sisters, Donna, Sherry, Karen and Denise and brothers, Arthur and Gilbert.

ABSTRACT

Rats receiving cocaine in a specific single compartment may express conditioned motor responses when subsequently exposed to these cues. In two-compartment procedures, rats can develop a conditioned place preference (CPP) to the drug-associated environment when given a choice between the two compartments on a drug-free test. Although the direct effects of stimulants on motor activity can be blocked by D_1 or D_2 receptor antagonists, the establishment and the expression of classically conditioned motor activity cannot be blocked by some dopamine antagonists. This thesis examines the role of a D_2 dopamine receptor antagonist, haloperidol, and L-type Ca^{2+} channel antagonist, nimodipine, in cocaine-conditioning of motor activity and rewarding effects, and examines the ability of cocaine to produce context-dependent behavioural sensitization. Studies indicate that horizontal and vertical motor activity may not be classically conditioned by stimulants. Hence, this issue is also considered. The first investigation of this thesis showed that, in a single-compartment procedure, establishment of cocaine-conditioned horizontal activity was blocked by the L-type Ca^{2+} channel antagonist, nimodipine. In addition, establishment of behavioural sensitization was context-dependent and attenuated by nimodipine. The results of the second investigation, with a single compartment procedure, indicate that expression of cocaine-conditioned horizontal activity may be blocked by co-treatment with haloperidol and nimodipine, and expression of behavioural sensitization may be blocked by nimodipine. The results from these two investigations show a dissociation between classically conditioned horizontal activity and behavioural sensitization, indicating that cocaine-sensitization may not be entirely under conditioned-

stimulus control. The third investigation showed that the conditioned reinforcing effects of cocaine, as measured by cocaine-CPP, may also be blocked by nimodipine. The fourth investigation also utilized a two compartment procedure, and it revealed that neither conditioning nor behavioural sensitization of horizontal and vertical activity may be adequately explained by classical conditioning, operant conditioning, or by a three-component model emphasizing a combination of context-dependency, context-independency and behaviour-specificity. Instead, a comprehensive visual behavioural analysis that encompasses important experiential and environmental factors involved in the behavioural changes which take place following chronic stimulant administration, may be necessary to understand the context-specific effects of cocaine.

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TABLE OF CONTENTS

Chapter 1. General Introduction 1

1.1. Cocaine 1

1.2. Dopamine Receptors 7

1.3. Stimulant-Induced Motor Activity and Neural Substrates 11

1.4. Rewarding Effects of Stimulants 13

1.4.1. Models of Drug Addiction 14

1.4.2. The Negative Reinforcement Model of Drug Addiction 14

1.4.3. The Positive Reinforcement Model of Drug Addiction 16

1.4.4. The Incentive-Sensitization (Neuroadaptationist) Model of Drug Addiction 17

1.4.5. Neural Substrates of Reinforcement 23

1.5. Behavioural Sensitization 25

1.5.1 Relationship of Behavioural Sensitization to Stimulant-Induced Psychoses and Schizophrenia 26

1.5.2. Neural Substates of Behavioural Sensitization 27

1.5.3. The Role of Classical Conditioning and Context-Specificity in Sensitization 31

1.6. Classical Conditioning of Psychomotor Stimulant Effects 32

1.6.1. Extinction of Conditioned Responses 34

1.6.2 Latent Inhibition of Classical Conditioning 35

1.6.3. Classical Conditioning of Motor Stimulant Effects 35

1.6.4. Neural Substrates of Classically Conditioned Motor Effects ... 36

1.6.5. Conditioned Reward-like Effects of Psychomotor Stimulants .. 39

1.6.6. Neural Substrates of Conditioned Reward-like Effects of

Psychomotor Stimulants 41

1.6.7 L-type Ca^{2+} Channels 43

1.7. Thesis Objectives 45

Chapter 2. Nimodipine and Haloperidol Attenuate Behavioural Sensitization to Cocaine But Only Nimodipine Blocks the Establishment of Conditioned Locomotion Induced by Cocaine 47

2.1. Introduction 47

2.2. Materials and Methods 48

2.2.1. Subjects 48

2.2.2. Drugs 49

2.2.3. Apparatus 49

2.2.4. Procedure 49

2.2.5. Statistics 50

2.3. Results 51

2.3.1. Experiment 1 51

2.3.2. Experiment 2 56

2.4. Discussion 59

Chapter 3. Effects of Nimodipine and/or Haloperidol on the Expression of Conditioned Locomotion and Sensitization to Cocaine in Rats 67

3.1. Introduction 67

3.2. Materials and Methods 67

3.2.1. Subjects 67

3.2.2. Drugs 67

3.2.3. Apparatus 68

3.2.4. Procedure 68

3.2.5. Statistics 70
3.3. Results 72
3.4. Discussion 74
Chapter 4. Nimodipine Prevents the Establishment of Cocaine-Conditioned Place Preferences 81
4.1. Introduction 81
4.2. Materials and Methods 82
4.2.1. Subjects 82
4.2.2. Drugs 83
4.2.3. Apparatus 83
4.2.4. Procedure 84
4.2.5. Statistics 85
4.3. Results 86
4.3.1. Conditioned Place Preferences 86
4.3.2. Cocaine Challenge Test 86
4.3.3. Horizontal and Vertical Activity 89
4.4. Discussion 89
Chapter 5. Classically Conditioned Motor Effects Do Not Occur with Cocaine in an Unbiased Conditioned Place Preferences Procedure 97
5.1. Introduction 97
5.2. Methods 100
5.2.1. Subjects 100
5.2.2. Drugs 101
5.2.3. Apparatus 101
5.2.4. Procedure 102

5.2.4.1. Experiment 1 102
5.2.4.2. Experiment 2 104
5.2.5. Statistics 104
5.3. Results 105
5.3.1. Conditioned Place Preferences 105
5.3.2. Horizontal and Vertical Activity 107
5.3.3. Horizontal Activity 107
5.3.4. Vertical Activity 113
5.3.5. Horizontal Activity in Groups 113
5.3.6. Vertical Activity in Groups 116
5.3.7. Extinction Period (Horizontal Activity) 116
5.3.7. Extinction Period (Vertical Activity) 121
5.4. Discussion 121
Chapter 6. General Discussion 133
6.1. Discussion 133
6.2. Conclusions 141
6.3. Clinical Implications 143
References 145

LIST OF TABLES

Table 1. Time in the drug compartment on the final habituation day 87

LIST OF FIGURES

Figure 1. Cocaine and major metabolites 3

Figure 2. Cocaine (5, 10 and 20 mg/kg) effects on locomotion 53

Figure 3. Cocaine-conditioned locomotion 54

Figure 4. Environment-specific cocaine-induced sensitization 55

Figure 5. Haloperidol, nimodipine and cocaine effects on locomotion: unpaired 57

Figure 6. Haloperidol, nimodipine and cocaine effects on locomotion: paired 58

Figure 7. Haloperidol, nimodipine and cocaine effects on conditioned locomotion: unpaired test day 60

Figure 8. Haloperidol and nimodipine effects on cocaine-conditioned locomotion: establishment 61

Figure 9. Acute effects of haloperidol, nimodipine and cocaine on conditioned locomotion 71

Figure 10. Locomotor activity induced by cocaine: photobeam interruptions vs % of control 73

Figure 11. Haloperidol and nimodipine effects on cocaine-conditioned locomotion: expression 75

Figure 12. Haloperidol and nimodipine effects on cocaine-sensitization: expression 76

Figure 13. Nimodipine effects on cocaine-CPP: establishment 88

Figure 14. Nimodipine effects on cocaine-CPP: cocaine challenge test 90

Figure 15. Nimodipine effects on cocaine-induced locomotion 91

Figure 16. Nimodipine effects on cocaine-induced rearing 92

Figure 17. Cocaine-CPP and extinction 106

Figure 18. Environmental and habituation effects on cocaine-induced locomotion and rearing 108-109

Figure 19. Cocaine-conditioning, -extinction and -sensitization tests: effects of habituation and environment (tunnel closed) 111-112

Figure 20. Cocaine-conditioning, -extinction and -sensitization tests: effects of habituation and environment (tunnel opened) 114-115

Figure 21. Extinction days for locomotion and rearing: habituated groups 118-119

Figure 22. Extinction days for locomotion and rearing: non-habituated groups 120

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine
5-HIAA	5-hydroxyindoleacetic acid
6-OHDA	6-hydroxydopamine
ANOVA	analysis of variance
α-MPT	alpha-methyl-*para*-tyrosine
CD	critical difference
C or COC	cocaine
COC-C	cocaine-closed
COC-O	cocaine-open
CPP	conditioned place preferences
CR	conditioned response
CS	conditioned stimulus
CS+	conditioned stimulus positive
CS-	conditioned stimulus negative
DSP4	N-(chloroethyl)-N-2-bromobenzylamine
G-protein	guanine nucleotide binding protein
H	haloperidol
ICSS	intracranial self-stimulation
IP	intraperitoneal
IVSA	intravenous self-administration
MDA	3,4-methylenedioxyamphetamine

MDMA 3,4-methylenedioxymethamphetamine

mRNA . . . . . messenger ribonucleic acid

N . . . . . nimodipine

n . . . . . number

p . . . . . probability

PHNO . . . . . (+)-4-propyl-9-hydroxynaphthoxazine

SEM . . . . . standard error of the mean

UCR . . . . . unconditioned response

UCS . . . . . unconditioned stimulus

V or VEH . . . . . vehicle

VEH-C . . . . . vehicle-closed

VEH-O . . . . . vehicle-open

CHAPTER 1. GENERAL INTRODUCTION

1.1. COCAINE

The central stimulant properties of cocaine have been known for centuries. When the Spanish Conquistadors arrived in Peru they found that the Incas attached enormous significance to the leaves of the plant *Erythroxylon coca*, for religious, mystical, social, stimulant and medicinal purposes (Fleming et al., 1990). The use of the coca plant was restricted to the ruling classes, although its religious significance and importance declined with the deterioration of the Incan civilization. The Spaniards pronounced coca to be sinful, and attempted to prevent its use among their Indian slaves. However, the Spaniards soon discovered that the Indians would work very hard under perilous conditions if they were allowed to use coca. Coca's stimulant effects were not discovered by the Europeans until the 1850's, when its principal constituent, cocaine, was isolated. The use of cocaine increased dramatically over the next few decades. Sigmund Freud initially believed that the drug could cure both medical and psychological problems, although he soon discovered that the drug could have addictive as well as other toxic side effects (Johanson and Fischman, 1989).Cocaine was first introduced to Western medicine (1884) as a local anesthetic and intense vasoconstrictor (Holmstedt et al., 1981). However, since the early 1970's cocaine abuse has reached epidemic proportions (Adams and Kozel, 1985), and its use may be nearly at peak levels today (Johanson and Schuster, 1995).

Cocaine is self-adminstered by humans in several ways, including nasally, intravenously and by smoking. Following nasal administration, peak plasma levels

occurred in approxmately 30 min, whereas intravenous administration (Johanson and Fischman, 1989), or administration by smoking resulted in peak plasma levels in about 4 min (Foltin and Fischman, 1991). Regardless of route of administration, the elimination half-life of cocaine was about 40 min (Johanson and Schuster, 1995), although some authors have reported half-lives as long as 90 min in humans (Jatlow, 1987), particularly in those who are homozygous for atypical cholinesterase (Jatlow, 1979). In rats, the motor stimulant effects of cocaine usually disappear within 30 to 120 minutes, depending on dose and route of administration, although intraperitoneal doses greater than 30 mg/kg may produce motor activity for over 2 h (Post, 1981; Lau et al., 1991). In addition, intraperitoneal injections produce higher activity levels than oral administration in rats (Lau et al., 1991). Half-lives in the rat are similar to those in humans (Lau et al., 1991), although half-life has been reported to be as low as 16 min in mice (Benuck et al., 1987).

Cocaine is a stereoselective compound, with (-)-cocaine the active enantiomer and (+)-cocaine having little or no CNS stimulant effects (Katz et al., 1990). Cocaine is metabolised by plasma cholinesterases into its two main metabolites, benzoylecognine and ecognine methyl ester. The only active metabolite of (-)-cocaine is norcocaine, and it is produced through metabolism of cocaine by cytochrome P450 enzymes (Figure 1). In a rat study, the half life of norcocaine was found to be about 25 min (Lau et al., 1991).

Genetics may play a role in cocaine abuse. Although there is little information available through human studies, animal studies indicate genetic differences in cocaine effects. For example, cocaine produces locomotor activity in "short sleep", but not in "long sleep" strains of mice (George and Ritz, 1990). Another study found that Fischer and Lewis strains of rat did not differ in locomotor activity or conditioned taste aversion,

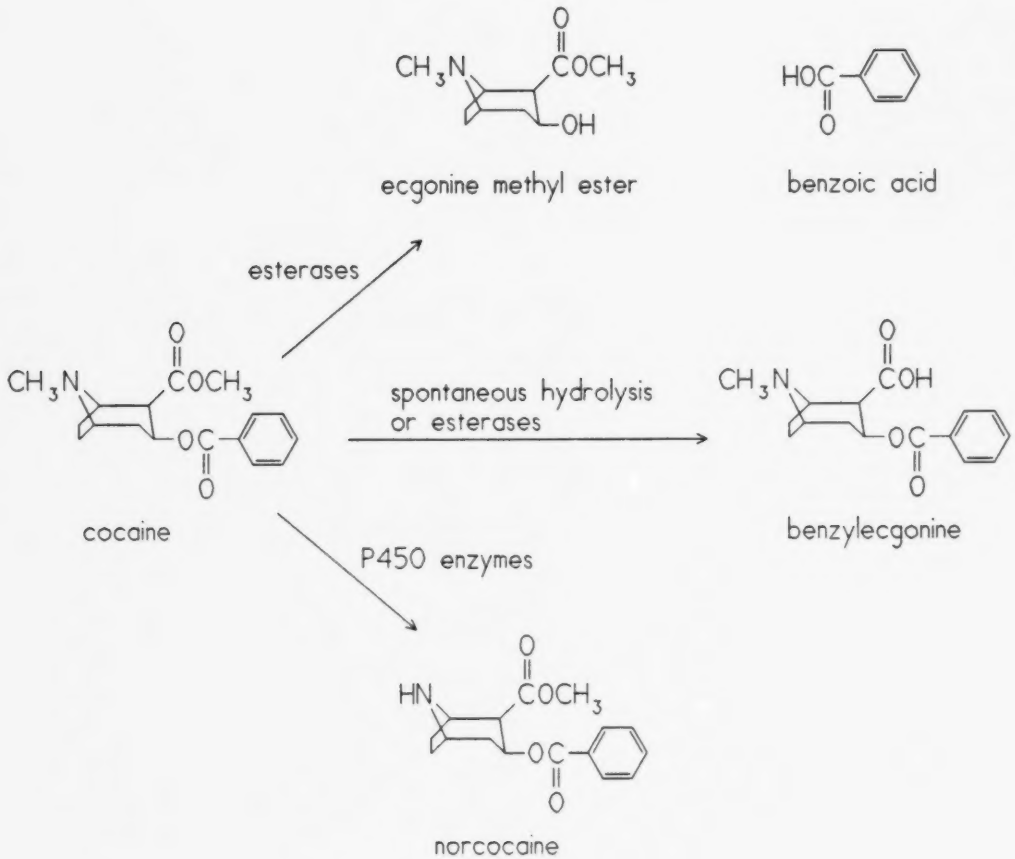

Figure 1: **Cocaine and major metabolites.**

although conditioned place preferences were greater in the Lewis strain, and behavioural sensitization did not occur in the Fischer strain (Kosten et al., 1993).

Central nervous system stimulant drugs fall into three main categories. These are the convulsants and respiratory stimulants such as picrotoxin and doxapram, respectively; the psychomotor stimulants such as cocaine, amphetamine, nicotine, and caffeine; and the psychotomimetic drugs such as mescaline and lysergic acid diethylamide (LSD) (Rang and Dale, 1991). This thesis is concerned with psychomotor stimulants in the cocaine and amphetamine class, which principally increase neurotransmission of the catecholamines, dopamine and noradrenaline (Rang and Dale, 1991). These drugs have high abuse liability (Cox et al., 1983), and are also important because they can result in stimulant-induced psychosis, a condition which is similar to paranoid schizophrenia (Angrist, 1983; Robinson and Becker, 1986).

Among other actions, cocaine and amphetamine enhance neurotransmission of dopamine, the principal neurotransmitter implicated in stimulant-induced behaviours (Robinson and Becker, 1986; Robinson and Berridge, 1993). Studies utilizing alpha-methyl-*para*-tyrosine (αMPT) and reserpine have helped to elucidate the differences between these two types of stimulants. αMPT is a specific inhibitor of tyrosine hydroxylase (McMillen, 1983), the rate limiting enzyme in catecholamine synthesis, and reserpine disrupts the vesicular uptake storage mechanism of the biogenic amines, irreversibly damaging the vesicle (Braestrup, 1977; Cooper et al., 1991).

Cocaine and similar stimulant drugs block the uptake of dopamine from the synaptic cleft and affect release from the Ca^{2+}-dependent, vesicular, reserpine-sensitive

dopamine pool (Fleming et al., 1990), as determined by uptake of [^{3}H]-dopamine (Ross, 1979), and by detection of dopamine overflow with *in vivo* voltammetry (Kelly and Wightman, 1987). Other drugs in this category include methylphenidate, amfonelic acid, pipradrol, GBR12909, and nomifensine (Scheel-Kruger, 1972; Braestrup, 1977; Ross, 1979; Clemens and Fuller, 1979; Van der Zee et al., 1980). In addition to a decrease in dopamine overflow following reserpine treatment, as determined by dopamine metabolites, the motor stimulant effects of these drugs are also blocked by administration of reserpine (Scheel-Kruger, 1972; Braestrup, 1977; Clemens and Fuller, 1979).

Amphetamine and its congeners principally affect dopamine release by an exchange-diffusion process (Braestrup, 1977; Clemens and Fuller, 1979; McMillen, 1983), although high doses of amphetamine can also block dopamine uptake (Ferris et al., 1972) and inhibit monoamine oxidase (Clarke, 1980). In the exchange-diffusion process, it is thought that a membrane-bound carrier binds the stimulant molecule (in the extracellular domain where the drug is highly concentrated) and a Na^+ ion, and is then transported across the membrane into the nerve terminal. The carrier can then bind a dopamine molecule (which is at higher concentrations within the terminal) and a Na^+ ion, and transport both of them out into the synaptic cleft (McMillen, 1983). Further support for a role of amphetamine in exchange diffusion comes from studies indicating that amphetamine decreases the concentration of the intracellular dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), due to its ability to block intracellular monoamine oxidase (Clemens and Fuller, 1979). In addition, amphetamine-induced motor activity is abolished by αMPT (Stolk and Rech, 1970; Tessel et al., 1975; Clemens and Fuller,

1979). This category of stimulant drug mainly affects dopamine release from the Ca^{2+}-independent, newly synthesized, αMPT-sensitive dopamine pool and includes other drugs such as methamphetamine, phenmetrazine, and pemoline (McMillen, 1983).

In addition to dopamine, psychomotor stimulants also affect other neurotransmitters. For example, cocaine also blocks uptake of norepinephrine (MacMillan, 1959; Hertting et al., 1961; Ritz et al., 1987, 1990) and serotonin (Ritz et al., 1987, 1990), although there does not appear to be any correlation between cocaine's reinforcing effects and its effects on these neurotransmitters (for review see Johanson and Schuster, 1995). However, it appears that noradrenergic systems are involved in the discriminative stimulus properties of cocaine. For example, in mice trained to discriminate the noradrenergic agonist nisoxetine, cocaine substitutes as a discriminative stimulus (Snoddy and Tessel, 1983). It has also been shown that the α-1 receptor agonist prazosin blocks the locomotor activating effects of cocaine (Snoddy and Tessel, 1985).

Amphetamine also affects noradrenergic and serotonergic systems. Amphetamine may induce a in release of [^{3}H]-norepinephrine from synaptosomes (McMillen, 1983), and amphetamine or methamphetamine administration reduces tryptophan hydroxylase activity, and cortical 5-HT and 5-HIAA (Peat et al., 1985). In addition, intrastriatal administration of the major metabolites of amphetamine, para-hydroxyamphetamine and para-hydroxy-norephedrine significantly decreased tryptophan hydroxylase activity and 5-HIAA concentrations (Matsuda et al., 1989). However, it is not believed that the reinforcing effects of amphetamine occur through norepinephrine or 5-HT (Robinson and Berridge, 1993; but also see NEURAL SUSTRATES OF REINFORCEMENT, below).

Although it is known that the behavioural effects of stimulant drugs principally occur through activation of dopamine neurotransmission (Angrist, 1983; Robinson and Becker, 1986), the dopamine system is quite complicated. The dopamine network includes several receptor subtypes (Seeman and Van Tol, 1994), as described below.

1.2. DOPAMINE RECEPTORS

There are two principal dopamine receptor subtype classifications in the human brain: D_1-like and D_2-like (Seeman and Van Tol, 1994). D_1-like subtypes include D_1 and D_5 receptors, and D_2-like subtypes include D_2, D_3, and D_4 receptors. Each of the two main dopamine receptor classifications can be further subdivided into variants of each subtype (Seeman and Van Tol, 1994). Dopamine receptors are characterized by seven transmembrane domains, and are coupled to guanine nucleotide binding proteins (G-proteins) (O'Dowd, 1993). D_1-like receptors stimulate adenylate cyclase and most D_2-like receptors inhibit adenylate cyclase (Kebabian and Calne, 1979). However, recent studies have shown that both subtypes interact with other second messenger systems (Rodrigues and Dowling, 1990; Morra et al., 1991), and there may be interactions between second messenger systems of D_1 and D_2 receptors (Schinelli et al., 1994).

Only one human D_1 receptor variant is known, although three variants of human D_5 have been found (Grandy et al., 1991; Nguyen et al., 1991). However, the amino acid sequences of the second and third D_5 receptors are only one third of the length of the original D_5, with the amino acid sequence terminating before the fourth transmembrane domain (Grandy et al., 1991; Nguyen et al., 1991a). The function of these so-called D_5-

pseudo-1 and D_5-pseudo-2 "receptors" is not known. The peptide sequence of the human D_1 receptor is 446 amino acids long (Sunahara et al., 1990), that of D_5 is 477, and D_5-pseudo-1 and -2 are both 154 amino acids long (Sunahara et al., 1991; Weinshank et al., 1991).

D_2 receptors are comprised of two main variants, D_2-short and D_2-long, with the difference being 29 additional amino acids in the third intracellular loop of D_2-long (Montmayeur et al., 1993). The third intracellular loop is thought to be important in G-protein coupling. The peptide sequence of D_2-short is 414 amino acids long, while that of D_2-long is 443 amino acids long. Three other variants of D_2-long also exist that are also 443 amino acids long (Montmayeur et al., 1993).

There are 5 main variants of the human D_3 receptor, including short (342 amino acids) and long (400 amino acids) forms (Snyder et al., 1991; Sokoloff et al., 1992; Nagai et al., 1993; Schmauss et al., 1993). The two latest forms are only 138 and 109 amino acids long, and stop after transmembrane domains two and three, respectively (Snyder et al., 1991). These forms are generated by alternative splicing which results in a premature stop codon (Nagai et al., 1993). Both forms have been found in expression systems used, and in brain (Sokoloff et al., 1992; Schmauss et al., 1993). So far, radioligand binding has not been demonstrated with these forms, and their function is unknown.

The D_4 receptor has many variants in the human (Van Tol et al., 1991, 1992; Lichter et al., 1993; Ashgari et al., 1994). Each variant has a different number of repeat units of sixteen amino acids in the third intracellular loop. Most humans (60%) have four of these repeats, and this is named the $D_{4.4}$ receptor. The $D_{4.7}$ receptor is present in about

14% of humans and $D_{4.2}$ is present in about 10%. Other D_4 variants are present in about 4% of humans, although $D_{4.0}$, $D_{4.1}$, and those with more than ten repeats have not been found. The peptide sequence length of D_4 receptors increases by 16 amino acids with each additional repeat, ranging from $D_{4.2}$ (387 amino acids) to $D_{4.10}$ (515) (Van Tol et al., 1991, 1992; Lichter et al., 1993; Ashgari et al., 1994). There are also at least 19 different types of repeat units, although the first and last repeats are identical in most cases (Lichter et al., 1993).

In the human brain, D_1-like receptors are more abundant and more widespread than D_2-like receptors (DeKeyser, 1993; Strange, 1993). D_1 receptors are found throughout cortical and limbic areas (DeKeyser, 1993), but are most heavily concentrated in the striatum, the nucleus accumbens, and the olfactory tubercle (Dearry et al., 1990). D_5 receptor messenger ribonucleic acid (mRNA) is present in much lower levels in these D_1-like areas, and is also present in the hippocampus and hypothalamus (Sunahara et al., 1991; Tiberi et al., 1991).

For D_2 receptors, the highest concentrations of mRNA are found in the ventral tegmental area and in the substantia nigra pars compacta, where cell bodies of dopaminergic neurons are found, although these are not the areas of highest receptor density (DeKeyser, 1993; Strange, 1993). D_2 mRNA is also found in the striatum, and in limbic regions such as the olfactory tubercle and nucleus accumbens. These are the terminal regions of dopaminergic cell bodies. D_2 receptors are found in highest concentrations in the striatum and nucleus accumbens, and in increasingly lower density in the globus pallidus, the substantia nigra, the amygdala, and the cerebral cortex,

respectively (O'Malley et al., 1990; Mansour et al., 1991; DeKeyser, 1993; Seeman et al., 1993).

The D_3 receptor and its mRNA are localized mainly to limbic areas of the brain such as the nucleus accumbens and olfactory tubercle, suggesting involvement with emotional behaviour (Sokoloff et al., 1990; Bouthenet et al., 1991). However, so far there is no evidence of linkage of the D_3 receptor gene to schizophrenia (Coon et al., 1993; Wiese et al., 1993). D_3 receptors have also been located in the putamen (DeKeyser et al., 1989a) and globus pallidus (DeKeyser et al., 1989a,b), areas important for motor activity, and were absent in the putamen of victims of Huntington's chorea (DeKeyser et al., 1989c).

The distribution pattern of D_4 receptor mRNA is distinct from other dopamine receptors. Most studies find D_4 receptors in D_2-rich areas such as the striatum, but higher levels of D_4 expression in limbic areas such as the frontal cortex, hippocampus, and amygdala (Mansour et al., 1991; Meador-Woodruff et al., 1991). The atypical antipsychotic drug, clozapine, binds at the D_4 receptor with 10 times higher affinity than at D_2 or D_3 receptors (Van Tol et al., 1991; Sokoloff et al., 1992), and does not produce pseudo-Parkinsonism or other extrapyramidal side effects such as dystonia or akathesia (Seeman, 1990; Gerlach, 1991). Clozapine and other atypical antipsychotic drugs also appear to result in a more significant improvement in the negative symtoms (eg. social withdrawal and flat affect) of schizophrenia than typical antipsychotics do (Kane et al., 1988; Seeman, 1990; Gerlach, 1991; Seeman and Van Tol, 1994). So far, however, there is no evidence of an association between schizophrenia and the D_4 receptor gene (Sommer

et al., 1993; Daniels et al., 1994).

Although molecular biological techniques have now determined that dopamine receptor subtypes D_3, D_4, and D_5 exist, there are few pharmacological agents that exist as yet that are highly specific for them (Cooper et al., 1991; Gerlach, 1991; Seeman and Van Tol, 1994). Hence, their role in behaviour is not clear.

1.3. STIMULANT-INDUCED MOTOR ACTIVITY AND NEURAL SUBSTRATES

Psychomotor stimulants result in an increase in the frequency and duration of some motor behaviours in animals (Post et al., 1981; Beninger and Herz, 1986; Martin-Iverson, 1991, Martin-Iverson and Fawcett, in press). D_1-like and D_2-like dopamine receptors interact to produce the motor stimulant effects of dopamine agonists (Clark and White, 1987). D_1-like agonists have little behavioural effect on their own, but can increase grooming behaviour in rats (Murray and Waddington, 1987). Horizontal and vertical activity, sniffing, head movements and other stereotyped behaviours produced by selective D_2-like agonists are attenuated by SCH23390 (Pugh et al., 1985), a selective D_1-like antagonist. Dopamine depletions with reserpine, αMPT, or reserpine + αMPT, attenuate the motor stimulant actions of selective D_2 agonists, but can be reinstated by selective D_1-like agonists such as SKF38393 (Braun and Chase, 1986; White et al., 1988; Dreher and Jackson, 1989). In addition, D_1-like agonists potentiate D_2-induced behavioural stereotypies (Mashurano and Waddington, 1986), and appear to be necessary for D_2-like agonists to produce the full spectrum of motor stereotypies (Barone et al., 1986).

Evidence suggests that cocaine and amphetamine increase motor activity mainly through increasing dopamine neurotransmission (Fishman et al., 1983). However, at least 90% of striatal dopamine must be depleted to abolish the locomotor response to cocaine or amphetamine (Creese and Iversen, 1975). Similarly, 6-OHDA-induced lesions of the nucleus accumbens, co-administered with desipramine, blocked amphetamine and cocaine-induced motor activity (Kelly and Iversen, 1976). Selective lesioning of dopamine or norepinephrine neurons with intracisternal 6-OHDA, via either co-administration of desipramine to protect norepinephrine neurons, or with amfonelic acid or GBR12909 to protect dopamine neurons, indicated that dopamine depletion, but not norepinephrine depletion, influences spontaneous motor activity (Luthman et al., 1989), and blocks amphetamine-induced motor activity (Creese and Iversen, 1973).

In addition, administration of D_2 receptor antagonists such as haloperidol, perphenazine, pimozide, spiramide, or trifluoperazine blocked amphetamine-induced locomotion (Hitzemann et al., 1971; Jackson et al., 1975; Handley and Thomas, 1978), while α- (Hasselager et al., 1972; Pijnenberg et al., 1975) or β-adrenoreceptor antagonists (Pijnenberg et al., 1975; Costall et al., 1976) had no effect. On the other hand, norepinephrine agonists, but not the direct dopamine agonist, apomorphine, enhanced amphetamine-induced motor activity (Handley and Thomas, 1978).

Administration of p-chlorophenylalanine, a potent 5-HT synthesis inhibitor, usually potentiates spontaneous (Volcier, 1969; Fibiger and Campbell, 1971; Borbely et al., 1973), and dopamine-induced motor activity (Mabry and Campbell, 1973; Segal, 1976). Similarly, electrolytic or 5,6- or 5,7-dihydroxytryptamine-induced lesions of raphé nuclei,

which decrease central levels of 5-HT and 5-HIAA, enhance spontaneous (Lorens et al., 1971;Grabowska, 1974) or amphetamine-induced motor activity (Breese et al., 1974).

Finally, recent work indicates that the role of dopamine in the initiation of motor activity is not as dominant as previously thought. Instead, glutamate and gamma-amino-butyric acid (GABA) are also important in regulation of motor activity (Graybiel, 1990; Kalivas, 1993; Carlsson, 1993). Thus, although the story is complicated, it appears that dopamine activation by psychomotor stimulant drugs is important in modulation of motor activity.

1.4. REWARDING EFFECTS OF STIMULANTS

The rewarding effects of psychomotor stimulant drugs have been determined through the use of 3 major experimental paradigms (Nestler, 1992). Self-administration and intracranial self-stimulation are operant paradigms where animals learn to perform a task in exchange for a drug injection or electrical stimulation. Conditioned place preferences (CPP) is a classical conditioning paradigm where animals learn to associate drug effects with a particular environment. In addition to these major paradigms, enhancement of conditioned reinforcement is also an important model of drug reward (Hill, 1970). In this paradigm, a tone is paired with administration of food to an animal several times. The animal is then placed back in the test environment with two levers available. Pressing one of the levers results in a tone, but pressing the other lever has no effect. Administration of a reinforcing drug with the initial pairings of the food and tone results in enhanced responding on the "tone lever" during the test.

1.4.1 MODELS OF DRUG ADDICTION

There are currently at least 3 models of drug addiction (Robinson and Berridge, 1993). These include the 1) negative reinforcement model, 2) the positive reinforcement model, and 3) the incentive-sensitization theory of addiction.

1.4.2 THE NEGATIVE REINFORCEMENT MODEL OF DRUG ADDICTION

The negative reinforcement model of addiction focuses on the withdrawal symptoms that result when drug use is discontinued (Stewart et al., 1984; Dackis and Gold, 1985; Wise and Bozarth, 1987; Koob et al., 1989; Jaffe, 1990; Nestler, 1992). Withdrawal symtoms in humans include psychological symptoms such as anxiety and depression, and physiological symptoms such as pain, body temperature, skin resistance, and heart rate (Wise and Bozarth, 1987; Childress et al., 1988; Gawin and Ellinwood, 1988). In animals, measures of withdrawal may include physical symptoms such as shaking (Wei et al., 1973, 1975), or direct signs of aversion such as escape responses (Wei et al., 1973, 1975) and conditioned place aversions (Bechara et al., 1987; Swerdlow et al., 1989). Addictive drugs such as cocaine and amphetamine that do not result in aversive physical symptoms are thought to act as negative reinforcers by alleviating unpleasant psychological symptoms resulting from withdrawal (Gawin and Ellinwood, 1988). According to the negative reinforcement theory, drug use is maintained to alleviate aversive symptoms resulting from withdrawal. A second view of the negative reinforcement model is that drugs are sometimes used for self-medication purposes (Khantzian, 1985). There are several problems with the negative reinforcement model.

(I) It has been noted that physical dependence (Wise and Bozarth, 1987) is not

necessary in rats, monkeys (Ternes et al., 1985; Schuster, 1990) or humans (Woods and Schuster, 1968; Lamb et al., 1991) for drugs to act as reinforcers, since both animals or humans will self-administer opioids in the absence of withdrawal symptoms or physical dependence, as determined by the absence of an effect of naloxone challenge.

(II) Maximal periods of drug self-administration do not correlate with maximal periods of withdrawal distress (Wise and Bozarth, 1987), even when the differences in withdrawal symptoms, which vary across drug classes from very mild to life threatening, are accounted for (Jaffe, 1992).

(III) Drugs such as clinical antidepressants, anticholinergics, and κ-opioid agonists produce withdrawal symptoms but are not addictive (Jaffe, 1992).

(IV) Relief of withdrawal does not effectively treat addiction (Wise and Bozarth, 1987).

(V) There is a high probability of relapse long after withdrawal symptoms have disappeared (Wikler, 1948; Siegel, 1988). Conditioned drug-associated effects are not responsible for this occurrence since at least a third of opiate addicts do not report drug-associated stimuli-induced withdrawal symtoms (Childress et al., 1988). In addition, there is a poor correlation between drug craving and withdrawal symptoms (Childress et al., 1988). Futhermore, animals do not self-administer drugs to alleviate conditioned withdrawal symptoms (Stewart et al., 1984).

(VI) The self-reported craving for alcohol (Meyer, 1988), cocaine (Childress et al., 1988; Jaffe et al., 1989), and opioids (Meyer, 1988; Ehrman et al., 1992) is often greatest immediately following drug administration, when the presence of withdrawal symptoms is at their weakest.

(VII) Animals will self-administer drugs directly into brain regions which do not produce withdrawal symptoms (ex. opiates into the ventral tegmental area) (Wise and Hoffman, 1992), and self-administration can be reinstated following extinction of responding in these animals by injecting these drugs into these same brain regions (Stewart et al., 1984).

For the reasons summarized above, it is now generally accepted that negative reinforcement is unnecessary for the development and maintenance of drug addiction.

1.4.3. THE POSITIVE REINFORCEMENT MODEL OF DRUG ADDICTION

The positive reinforcement view of addiction suggests drugs are self-administered because of the euphoric state they produce, rather than an aversive state that they alleviate (Stewart et al., 1984; Wise and Bozarth, 1987). However a number of findings suggest that the subjective pleasurable effects of drugs may not be necessary or sufficient to produce drug addiction.

(I) There is not a clear relationship between the ability of a drug to produce euphoria and addictive potential, and many addictive drugs initially produce dysphoric states (Robinson and Pritchard, 1992). To most people, including addicts, the negative consequences of continued drug use, such as loss of health, friends, job, family, etc. far outweigh the pleasure derived from drugs (Falk et al., 1983; Robinson and Berridge, 1993).

(II) A positive reinforcement theory of addiction cannot adequately explain drug craving or relapse elicited by environmental cues, since conditioned "highs" are much less frequent than conditioned "cravings" or conditioned withdrawal-like signs (Childress et al., 1988; O'Brein et al., 1992), suggesting that conditioned "cravings" are dissociable

from conditioned "highs" (Robinson and Berridge, 1993). In addition, the explicit memory of past pleasure from drug use cannot explain relapse, since an addict could not possibly have abstained for months or even years without remembering drug experiences many times. Therefore, why should the memory of a drug experience suddenly trigger a relapse (Robinson and Berridge, 1993)?

(III) Drug self-administration can be maintained in the absence of subjective pleasure. For example, "ex" opiate addicts would lever press for an injection of a low dose of morphine, despite the fact that 4 out of 5 subjects could not distinguish the morphine injection from placebo (Lamb et al., 1991). A similar dissociation between the affective and reinforcing properties of drugs have been reported in cocaine studies with humans (Fischman and Foltin, 1992), and in rat studies (White et al., 1977; Martin et al., 1991).

The findings summarized above suggest that positive reinforcement theories cannot adequately explain drug addiction.

1.4.4. THE INCENTIVE-SENSITIZATION (NEUROADAPTATIONIST) MODEL OF DRUG ADDICTION

The incentive-sensitization theory of drug addiction suggests that the repeated intermittent use of drugs can result in incremental and persistent changes in a neural system that mediates craving for a drug (Robinson and Berridge, 1993). This neural system is responsible for the attribution of incentive salience, and not pleasure, to stimuli. Incentive salience refers to the attractiveness, or the ability of a set of external stimuli, events, places, and their mental representations to capture attention (Robinson and Berridge, 1993). There are several criteria that must be met in order for this theory to be valid.

(I) A common neural system must be affected by many addictive drugs. Although addictive drugs such as amphetamine, cathinone, cocaine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), methylphenidate, ethanol, fentanyl, methadone, morphine, nicotine, and phencyclidine result in many different neurochemical and behavioural effects (Robinson and Berridge, 1993), there is evidence that all of these drugs increase mesolimbic/mesocortical dopamine neurotransmission (DiChiara and Imperato, 1988; Wise and Bozarth, 1987; Wise, 1987,1988).

(II) Repeated administration of addictive drugs should sensitize a common neural system in a gradual and incremental fashion. Intermittent administration of low to moderate doses of psychomotor stimulants such as cocaine and amphetamine can result in behavioural sensitization of the locomotor activating effects of these drugs (Robinson and Becker, 1986). In humans, the only comparable effect that has been characterized is sensitization to the psychotogenic effects of stimulants (Connell, 1958; Angrist, 1983; Robinson and Becker, 1986). Drugs such as opioids (Babini and Davis, 1972; Joyce and Iversen, 1979), nicotine (Benwell et al., 1992; Clarke, 1990), phencyclidine (Greenberg and Segal, 1985; Iwanmoto, 1986), ethanol (Crabbe et al., 1982; Cunningham and Noble, 1992), and MDMA (Spanos and Yamamoto, 1989) can also result in behavioural sensitization of locomotor activity in animals. It has been well established that the integrity of the mesotelencephalic dopamine system is important in the psychomotor activating effects of these drugs (Wise, 1987). Activation of the mesotelencephalic dopamine system is necessary to induce sensitization. For example, morphine-, amphetamine-, or cocaine-

induced sensitization can be blocked by dopamine antagonists (Kuczenski and Leith, 1981; Vezina and Stewart, 1989; Weiss et al., 1989; Hamamura et al., 1991), and administration of amphetamine or morphine directly into the ventral tegmental area (Kalivas and Stewart, 1991), or amphetamine into the nucleus accumbens (Kolta et al, 1989; Paulson and Robinson, 1991) induces sensitization. In addition, behavioural sensitization results in changes in mesotelencephalic dopamine activity (Robinson and Becker, 1986; Kalivas and Stewart, 1991; White and Wolf, 1991). For instance, *in vivo* microdialysis studies with amphetamine (Ichikawa, 1988; Patrick et al., 1991; Wolf et al., 1993) or application of amphetamine (Vezina and Zurakowski, 1991), cocaine (Akimoto et al., 1989; Pettit et al., 1990), methylphenidate (Kolta et al., 1985), morphine (Kalivas and Stewart, 1991), nicotine (Benwell et al., 1992), B-phenylethylamine (Kuroki et al., 1990), or ethanol (Benjamin et al., 1992), following cross sensitization between different drugs (Kalivas et al., 1988; Kazahaya et al., 1989; Akimoto et al., 1990), or following stress (Robinson et al., 1985) has shown that enhanced dopamine release occurs in response to drug challenge.

(III) Sensitization-induced neuroadaptations should be long lasting. Administration of amphetamine, cocaine, methylphenidate, or morphine can produce sensitization to a challenge injection of these drugs which is present weeks or even months later (Shuster et al., 1975; Browne and Segal, 1977; Shuster et al., 1982; Peris and Zahniser, 1987).

(IV) The expression of sensitization-induced neuroadaptations should be amenable to conditioned stimulus control. It has been shown that the drug-associated environment can come to elicit drug-like effects in the absence (Hinson and Poulos, 1981; Schiff, 1982; Stewart and Badani, 1993) or presence (Post et al., 1981; Vezina and Stewart, 1984;

Stewart and Vezina, 1991) of the drug, although sensitization is not always context-dependent (Robinson and Becker, 1986; Vezina and Stewart, 1990; Stewart and Vezina, 1991). In addition to conditioned stimulus control, environmental stimuli associated with drugs can also influence the response to other non-drug incentive stimuli (Mitchell and Stewart, 1990). For example, male rats trained with morphine in the drug-associated environment and then subsequently exposed to female rats, had more female-directed behaviours, such as anogenital sniffing and mounting, than home cage or vehicle controls. In other words, the female appeared to be a more salient incentive stimulus in the morphine-associated environment in test males. These results suggest that sensitization to drugs may change neural systems that modulate incentive properties of both drug-associated stimuli and natural incentives.

(V) Mesotelencephalic dopamine plays an important role in incentive motivation. Incentive motivation is a psychological concept of how incentives are developed (Bindra, 1978). According to this theory, incentive motivation is the principle mechanism controlling behaviours toward natural incentives such as food, water, sex, etc., and towards artificial incentives such as the effects of self-administered drugs (Bindra, 1978). Incentive motivation directed toward a particular set of external stimuli may be the result of a three stage process which results in salience attribution (see VI, below). Drive reduction and opponent processes are other examples of potential mechanisms that might control behaviour independent of incentive processes (Toates, 1986). Studies indicate that the dopamine system is important in mediating the incentive motivational effects of drugs, food, sex, and other natural incentives (Beninger, 1983; Fibiger and Phillips, 1986; Wise

and Bozarth, 1987; Hoebel, 1988; Robbins et al., 1989; Everitt, 1990; White and Milner, 1992). Signals predicting the availability of food, water, or a sexual partner activate dopamine neurotransmission in the nucleus accumbens (Church et al., 1987; Hernandez and Hoebel, 1988; Pleim et al., 1990; Damsma et al., 1992; Young et al., 1992). Together with the data from studies with addictive drugs (DiChiarra and Imperato, 1988), and the fact that dopamine antagonism can attenuate motivational properties of natural incentives and of addictive drugs (Wise, 1982), these results suggest that the common neural currency of incentives is mesotelencephalic dopamine neurotransmission.

(VI) The effects of dopamine are on incentive salience, not pleasure. It has been suggested that incentive motivation results from the ability of a drug or a natural incentive to produce pleasure, associative learning, and the attribution of incentive salience to external events and their representations (Berridge, 1991; Berridge and Valenstein, 1991). The difference between "wanting" and "liking" are important in incentive motivation, and it appears that separate neural processes underlie each component (Bindra, 1978; Toates, 1986). Incentive salience may result in the experience of "wanting" (Berridge, 1991; Berridge and Valenstein, 1991). This theory suggests that salience attribution is a specific psychological process that is normally activated in conjunction with pleasure (liking) and associative learning in the creation of new incentives. This three stage process successively involves 1) pleasure ("liking"), 2) associative learning, and finally, 3) incentive salience. Incentive salience transforms the sensory features of the incentive stimulus into a more salient stimulus which commands attention, becomes "wanted", and thereby directs behaviour toward the incentive, i.e., initially neutral stimuli can become conditioned

incentives. There are three lines of evidence which suggest that mesotelencephalic dopamine neurotransmission mediates incentive salience and not pleasure. **(i)** Dopamine antagonists do not decrease and dopamine agonists do not increase the sensory pleasure of tastes as measured by hedonic reactions in the taste reactivity paradigm, although they respectively decrease or increase incentive value (Treit and Berridge, 1990). Examples of strongly positive (hedonic) reactions to taste are paw licking, lateral (non-rhythmic) tongue protrusions, and rhythmic tongue protrusions along the midline. Neutral or compromise responses are mouth movements or passive drip from the mouth. Examples of strongly aversive reactions to taste in rats include gapes (large opening of the mandible and retractions of lower lip), chin rubbing (lowering mouth to floor and pushing forward), face washing (single wipe with the forepaws or a bout of several wipes), forelimb flails (shaking forelimb at greater than 60 Hz), headshaking (greater than 60 Hz), and others (Treit and Berridge, 1990). Depletion of nucleus accumbens and caudate dopamine by 6-OHDA lesions does not decrease hedonic evaluation of tastes, even though it abolishes motivation to eat and motivation for other natural incentives (Berridge and Valenstein, 1991). Electrical stimulation of the lateral hypothalamus, an area which results in activation forebrain dopamine projections, does not increase hedonic value of food. **(ii)** Electrophysiological experiments in animals suggest that dopamine neurons discharge to new orienting stimuli, but these neurons soon habituate and do not discharge when the animal actually eats food (Strecker et al., 1983). **(iii)** In rat studies with high speed chronamperometry (Gratton et al. 1992; Kiyatkin et al., 1992), dopamine release was increased at the time that a response was initiated and rapidly decreased following drug

infusion, consistent with the view that dopamine mediates incentive salience attributed to a drug-associated stimulus (the lever). Thus, the incentive-sensitization theory appears to be the most comprehensive theory of drug addiction, since it encompasses some of the aspects of the negative and the positive reinforcement models, and includes aspects that the first two theories overlook.

1.4.5. NEURAL SUBSTRATES OF REINFORCEMENT

Psychomotor stimulants such as cocaine and amphetamine are thought to activate the so-called reward systems of the brain, and thereby reinforce drug use (Pickens and Harris, 1968; Yokel and Pickens, 1973, 1974; Wise and Bozarth, 1987; DiChiara and Imperato, 1988; Robinson and Berridge, 1993). These reward systems include the mesolimbic/mesocortical dopamine network (Wise and Bozarth, 1987; DiChiara and Imperato, 1988; Koob and Bloom, 1988; Robinson and Berridge, 1993). More specifically, dopaminergic cell bodies project from the tegmentum to the striatum, the nucleus accumbens, and various cortical areas (Angevine and Cotman, 1981; Nestler, 1992). These tegmental areas are composed of the substantia nigra pars compacta (in area A9), and medial to this, the ventral tegmental area (in area A10) (Angevine and Cotman, 1981; Nestler, 1992). The compacta cell bodies project to the striatum, and the ventral tegmental cell bodies project to various cortical areas and to the nucleus accumbens (Angevine and Cotman, 1981). The ventral tegmental area and nucleus accumbens appear to be more important in reward-related processes than do the substantia nigra and striatum (Angevine and Cotman, 1981).

Amphetamine self-administration occurs with injections into the nucleus accumbens, but not into the caudate nucleus, and this anatomic dissociation has been reported consistently (Roberts et al., 1977; Pettit et al., 1984). Similar to amphetamine (Pickens et al., 1975), intravenous self-administration (IVSA) of cocaine was blocked by dopamine D_2 receptor antagonists (Ettenberg et al., 1982), and by depletion of nucleus accumbens dopamine with 6-OHDA (Roberts and Koob, 1982; Pettit et al., 1984; Zito et al., 1985), but not by depletion of frontal cortical dopamine (Martin-Iverson et al., 1985).

Intracranial self-stimulation (ICSS) with apomorphine is also blocked following dopamine depletion in the nucleus accumbens with 6-OHDA (Strecker et al., 1982). 6-OHDA lesions of the substantia nigra (Fibiger et al., 1976) or ventral tegmetal area (Phillips and Fibiger, 1978) suppress ICSS, even when noradrenergic terminals are spared, whereas depletions of norepinephrine does not suppress ICSS when electrodes are placed in the locus coeruleus, hippocampus, or olfactory bulb (Clavier et al., 1976).

Addictive drugs (DiChiara and Imperato, 1988; Hernandez and Hoebel, 1988) and natural reinforcers such as food (Hernandez and Hoebel, 1988), sex (Pleim et al., 1990; Damsma et al., 1992), and availability of water (Young et al., 1992), activate brain dopamine systems in the nucleus accumbens, as measured by microdialysis. Animals will work for electrical stimulation (Olds and Milner, 1954) or for microinjections of addictive drugs into the lateral hypothalamus, the medial prefrontal cortex, the nucleus accumbens, and the ventral tegmental area (Olds and Williams, 1980; Bozarth and Wise, 1981; Olds, 1982; Watson et al., 1989; Nestler, 1992; Wise and Hoffman, 1992). In addition, motivational properties of natural reinforcers and of addictive drugs are attenuated by

dopamine antagonists or by decreasing dopamine activity in rats (Jonsson et al., 1971; Wise, 1982), and application of 6-OHDA to the medial forebrain bundle results in aphagia in rats (Ungerstedt, 1971).

The effects of stimulants on neurotransmitters other than dopamine are probably not as important as those on dopamine for their reinforcing effects. Motivational properties of natural reinforcers and of self-administered drugs are not decreased in rats by norepinephrine antagonists (Jonsson et al., 1971). With the exception of bupropion and nomifensine, which have indirect dopamine agonist properties (Braestrup, 1977; Ferris et al., 1981), there is no evidence in animal studies of conditioned place preferences (Martin-Iverson et al., 1985; Swerdlow et al., 1989) or self-administration (Roberts and Goeders, 1989) resulting from antidepressant drug administration, although most antidepressants enhance the neurotransmission of norepinephrine or serotonin or both (Baker and Greenshaw, 1989).

1.5. BEHAVIOURAL SENSITIZATION

As summarized above, the repeated administration of psychomotor stimulants to rats can result in a gradual augmentation of the behavioural effects of the drug known as behavioural sensitization (Robinson and Becker, 1986). Included are behaviours such as locomotion, rearing, head movements, and with time, stereotyped behaviours such as licking and gnawing also emerge (Angrist, 1983; Segal and Schuckit, 1983; Robinson and Becker, 1986; Lyon, 1991).

1.5.1. RELATIONSHIP OF BEHAVIOURAL SENSITIZATION TO STIMULANT-INDUCED PSYCHOSES AND SCHIZOPHRENIA

Some researchers believe that behavioural sensitization is related to stimulant-induced psychoses in humans (Angrist, 1983; Robinson and Becker, 1986). Stimulant-induced psychoses are clinically very similar to the active psychotic phase of paranoid schizophrenia (Connell, 1958; Griffith et al., 1968; Angrist, 1983). Amphetamine psychosis has been the best characterized (Connell, 1958; Griffith et al., 1968; Angrist, 1983), although similar characteristics have been noted with cocaine psychosis (Angrist, 1983; Manschreck et al., 1987). Hallucinations and delusions are prominent both in stimulant and in schizophrenic psychosis (Connell, 1958; Griffith et al., 1968; Angrist, 1983). Flattening of affect has also been observed in some patients with amphetamine psychosis, although this effect usually occurred following long term use and was associated with a high doses of amphetamine (Griffith et al., 1968; Angrist and Gershon, 1970). Thought disorders have also been observed with high doses amphetamine, but are not common (Angrist, 1983).

However, there are differences between stimulant-induced psychoses and paranoid schizophrenia (Angrist, 1983; Cox et al., 1983). For example, the predominant hallucinatory experience with psychomotor stimulant psychoses is visual, whereas auditory hallucinations are the most common in schizophrenics (Cox et al., 1983). In addition, tactile (Cox et al., 1983) and olfactory (Angrist, 1983; Cox et al., 1983) hallucinations are quite common in stimulant psychoses but rarely occur with schizophrenic psychoses. Also unlike cases of schizophrenic psychoses, victims of stimulant-induced psychoses usually retain full memory, clear consciousness, and orientation with respect to time, place, and

self (Cox et al., 1983). Often, stimulant-psychotics are violent, whereas paranoid schizophrenics are not usually violent (Cox et al., 1983). Finally, once psychomotor stimulants have been excreted, psychoses usually disappear (Angrist, 1983; Cox et al., 1983). On the other hand, schizophrenics who take stimulants may not recover from a psychotic episode for a long time (Cox et al., 1983).

The most widely accepted theory regarding the neural substrates of schizophrenia is that there is increased neurotransmission in central systems involving the neurotransmitter dopamine (Lieberman et al., 1987; Seeman, 1990; Cooper et al., 1991; Seeman et al., 1993). This theory is supported by observations that the average clinical doses of antipsychotic medications is correlated with their ability to bind to dopamine D_2-like dopamine receptors (Seeman et al., 1974; Enna et al., 1976; Seeman and Van Tol, 1994). Further support for the dopamine theory of schizophrenia comes from the observations that stimulants intensify psychotic symptoms in schizophrenics and many victims of Parkinson's disease develop psychotic reactions in response to drug therapy with dopamine precursors or dopamine mimetics (Post, 1981; Angrist, 1983). In addition, methylphenidate, which may be the most potent psychotomimetic (Lieberman et al., 1987), induces an information processing deficit in normal volunteers that is similar to that found in schizophrenics not experiencing a psychotic episode (Braff and Huey, 1988). The exact nature of this dopamine involvement in stimulant psychoses and schizophrenia has not yet been determined.

1.5.2. NEURAL SUBSTATES OF BEHAVIOURAL SENSITIZATION

The neurochemical substrates of behavioural sensitization are not completely understood. Although it has been well established that the dopamine system is involved

in sensitization (Angrist, 1983; Robinson and Becker, 1986; Robinson and Berridge, 1993), no consistent changes in pre- or post-synaptic receptors (Conway and Uretsky, 1982), in spontaneous dopamine release (Kuczenski and Leith, 1981), or in dopamine synthesis, concentration or metabolites (Kuczenski and Leith, 1981; Nishikawa et al., 1983) has been reported. However, there does appear to be enhanced *in vitro* amphetamine-, KCl-, or electrical field-stimulated release of dopamine following chronic intermittent *in vivo* treatment with amphetamine (Antelman et al., 1980; Robinson and Becker, 1982; Robinson et al., 1982, 1988; Kolta et al., 1985a,b; Casteneda et al., 1988). Although these results have been confirmed *in vivo* in some studies with microdialysis (Akimoto et al., 1989,1990; Kazahaya et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990; Parsons and Justice, 1993), others have not reported increases in extracellular dopamine levels following sensitization to cocaine or amphetamine (Hurd et al., 1989; Kuczenski and Segal, 1990; Segal and Kuczenski, 1992a,b). It has been suggested that the reason for this discrepancy is that although behavioural sensitization is present regardless of the time following withdrawal, increases in extracellular dopamine levels depends on the number of days since withdrawal (Kalivas and Duffy, 1993). Therefore, enhancement of extracellular dopamine release may not be necessary for behavioural sensitization. It has not been reported that the absence of enhancement of stimulated dopamine release occurs after stimulant regimes which do not produce sensitization, although one *in vitro* study observed an increase in stimulated dopamine release following intermittent cocaine administration that produced behavioural sensitization, but not after continuous cocaine administration that produced tolerance (King et al., 1993).

Burger and Martin-Iverson (1994) have reported that intermittent daily cocaine produces behavioural sensitization, while equivalent quantities of cocaine administered continuously produce tolerance. Sensitization was blocked by co-treatment with the dihydropyridine L-type Ca^{2+} channel antagonist nimodipine, and this was accompanied by increased occupation of D_1 and D_2 receptors in the striatum and nucleus accumbens in rats displaying sensitization, but not in those displaying tolerance (Burger and Martin-Iverson, 1994). When a 10-day withdrawal period separated a challenge injection from the last treatment day, this relationship between sensitization and receptor occupation no longer existed. Pretreatment with nimodipine still reduced the increase in receptor occupation produced by cocaine, but did not block sensitization nor did it result in a difference in receptor occupation between sensitized and tolerant rats. Therefore, the relationship between increased dopamine release and sensitization is unclear.

It has been suggested that stimulant-induced sensitization may rely on a transient increase in activation of D_1 receptors together with activation of D_2 receptors (Martin-Iverson et al., 1988b). Sensitization to systemic injections of amphetamine was attenuated by injection of the D_1 receptor antagonist SCH23390 into the ventral tegmental area or the substantia nigra of rats (Stewart and Vezina, 1989). Furthermore, it has been shown that D_1 receptor activation is important in the establishment of apomorphine- (Mattingly et al., 1991) or amphetamine-induced sensitization (Drew and Glick, 1990). Finally, repeated cocaine administration increases the sensitivity of neurons to a D_1 but not to a D_2 receptor agonist (Henry and White, 1991). Thus, there is some support for the suggestion of a D_1/D_2 receptor interaction in behavioural sensitization.

It has been reported that steroid hormones can result in psychoses in humans (Lewis and Smith, 1983; D'Orban, 1989). The establishment of amphetamine-induced behavioural sensitization in rats can be blocked by injections of antiserum to corticotrophin-releasing factor (Cole et al., 1990), and chronic cocaine downregulates corticotrophin releasing factor receptors (Goeders et al., 1990). Adrenalectomy blocks the nocturnal sensitization to amphetamine, and this effect can be reversed by dexamethasone but not corticosterone, indicating a role for the glucocorticoid II receptor (Rivet et al., 1989). Central administration of corticotrophin releasing factor (Cador et al., 1993) or intermittent systemic administration of corticosterone (Deroche et al., 1992; Pauly et al., 1993) induces sensitization to amphetamine. These results indicate that the steroid endocrine system may play a role in stimulant-induced sensitization. In addition, the development of amphetamine, apomorphine, or cocaine-induced sensitization was blocked by a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK801 (Wolf and Khansa, 1991; Druhan et al., 1993; Wolf and Jeziorski, 1993). Repeated cocaine administration also decreases the concentration of G-proteins in the cell body and terminal regions of the mesolimbic dopamine system (Nestler et al., 1990; Striplin and Kalivas, 1993), and administration of an inhibitor of $G_{i\alpha}$-protein, pertussis toxin, enhances cocaine-induced locomotor activity (Steketee et al., 1991).

Thus, a number of factors have been proposed to mediate behavioural sensitization to psychomotor stimulant drugs. Further investigation will be required to determine if one mechanism is crucial, or whether there is an interaction among several mechanisms.

1.5.3. THE ROLE OF CLASSICAL CONDITIONING AND CONTEXT-SPECIFICITY IN SENSITIZATION

Stimulant-induced sensitization may be a function of at least two processes, a non-associative (Stewart and Vezina, 1991; Szechtman et al., 1993) and an associative process (Tilson and Rech, 1973; Post et al., 1981; Weiss et al., 1989, Stewart and Vezina, 1991; Willner et al., 1992; Stewart and Badani, 1993; Szechtman et al., 1993). For example, when cocaine (Post et al., 1981) or amphetamine (Tilson and Rech, 1973; Stewart and Vezina, 1991) administration is paired with a specific environment, sensitization resulting from a challenge injection of the conditioning stimulant is usually strongest in the stimulant-associated environment, thereby implicating a classical conditioning component to sensitization. Following extinction of amphetamine-conditioned horizontal and vertical activity, however, a challenge injection of amphetamine resulted in sensitization of horizontal but not vertical activity in rats (Stewart and Vezina, 1991). Willner et al. (1992) found that when rats were conditioned with the direct D_2/D_3 agonist quinpirole in a running wheel and then placed in a runway following a quinpirole challenge injection, sensitization was observed. In another group of rats conditioned in a locked running wheel, a quinpirole challenge did not result in sensitization in the runway, thus suggesting that sensitization was behaviour-specific, or operantly conditioned. In another study, Szechtman et al. (1993) calculated that quinpirole-induced sensitization may be approximately 50% context-specific, 30% behaviour-specific, and 20% context-independent.

However, behavioural sensitization also depends upon several other parameters, including: drug dosage (Reith, 1986; Robinson and Becker, 1986; Mattingly et al., 1988); injection schedule (Hiroi and White, 1989; Damianopoulos and Carey, 1993); route of administration (Dow-Edwards et al., 1989); behavioural measure (Post et al., 1981; Barr et al., 1981), structural features of the test environment (Post et al., 1981; Beck et al., 1986), previous experience with the environment (Mazurski and Beninger, 1988), genetics (Shuster et al., 1977) and circadian rhythms (Martin-Iverson et al., 1990a,b; Martin-Iverson, 1991).

1.6. CLASSICAL CONDITIONING OF PSYCHOMOTOR STIMULANT EFFECTS

It has now been well established that stimuli associated with the effects of a drug can be classically conditioned (Pickens and Crowder, 1967; Wikler and Pescor, 1967; Schiff, 1982). In classical conditioning studies with drugs, the drug is the unconditioned stimulus (UCS) and the drug effect is the unconditioned response (UCR). Drug-associated cues serve as conditioned stimuli (CS (or CS+)) for the conditioned response (CR) to a drug, and repeated drug administration in the presence of the same set of predrug stimuli leads to the acquisition of a CR. Conditioning is demonstated by the development of a CR to the CS alone, and is attributed to the simultaneous pairing of the CS and the UCS. Drug effects can be conditioned to environmental stimuli with many drugs, and this effect appears to be a function of the ability of these drugs to enhance dopamine neurotransmission (Robinson and Berridge, 1993).

Drug tolerance can be conditioned according to the Pavlovian paradigm with morphine (Siegel, 1975, 1977). CRs are often opposite or compensatory to the direct UCR of the drug. This results in an attenuation of the drug effect. A description of this effect is encompassed in the opponent process theory of drug addiction (Solomon and Corbit, 1974; Solomon, 1980). For example, according to the opponent process theory, when circumstances produce strong emotions, the opposite emotions are also strongly felt. The initial feeling that a person has with a potential drug of abuse is euphoria, but when the effect of the drug wears off, the user may feel "bad", or depressed. With chronic use of the drug, the negative reinforcing effects of the drug grow stronger, and a stronger stimulus (more drug) is required to get the same level of positive emotion that was initially experienced. In animal studies, when morphine experienced (morphine tolerant) rats were exposed to morphine-predictive stimuli, they showed tolerance to the drug's analgesic properties, but not in the presence of novel stimuli (Siegel et al., 1978; 1981). Thus, drug tolerance was classically conditioned to the initial environment.

The repeated use of psychomotor stimulants can result in the conditioning of drug effects to environmental stimuli also. Exposure of humans (O'Brien, 1975; Childress et al., 1987; O'Brien et al., 1988; Muntaner et al., 1989) or other animals (deWitt and Stewart, 1981; Beninger and Hahn, 1983; Hiroi and White, 1989) to drug-associated environmental cues has been shown to induce stimulant-like euphoric, arousing, and physiological effects even in the absence of the drug. Abstinent addicts returning home from 28 day detoxification programs reported a "high" in response to people, places, or other cues formerly associated with the drug (O'Brien et al., 1988; Childress et al., 1987). Such stimuli can also elicit physiological effects such as a decrease in skin temperature

(O'Brien et al., 1988), and an increase in heart rate and blood pressure (Muntaner et al., 1989). In addition, drug-associated effects can trigger a "craving" for the drug (O'Brien et al., 1988). It has been suggested that these conditioned effects may be very important in producing "drug craving" in addicts (O'Brien et al., 1988; Muntaner et al., 1989; Robinson and Berridge, 1993), and may maintain drug addiction even during treatment and detoxification therapies (Kang et al., 1991).

1.6.1. EXTINCTION OF CONDITIONED RESPONSES

When a CS is explicitly paired with the absence of the UCS, extinction of the CR results (Mackintosh, 1974). For example, methylphenidate-CPP was extinguished following 3 pairings of rats with the methylphenidate-associated environment in the absence of methylphenidate (Mithani et al., 1986). Similarly, in an operant conditioning procedure, lever pressing for amphetamine infusions extinguished once the infusions were no longer available following lever presses (Pickens and Harris, 1968). Depletion of dopamine or dopamine receptor blockade has an effect on operant responses that is similar to extinction trials (Pickens and Harris, 1968; Wilson and Schuster, 1972; Yokel and Wise, 1975, 1976; de Witt and Wise, 1977). Administration of nimodipine also blocks cocaine-CPP (Pani et al., 1991a), and this probably occurs through a reduction of dopamine neurotransmission (Pani et al., 1990a,b, 1991b). Similar to extinction of operant responding or of stimulant-CPP, some behaviours that appear to be classically conditioned with cocaine (Barr et al., 1983) or amphetamine (Pickens and Crowder, 1967) in rats, or cocaine "craving" in humans (O'Brien et al., 1988), can be extinguished. However, cocaine-conditioned horizontal and vertical activity did not extinguish (Barr et al., 1983).

Similar to the case for operant conditioning, the establishment of amphetamine- (Beninger and Hahn, 1983; Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1991, 1992a,b) or cocaine-"classically conditioned" locomotion (Beninger and Herz, 1986) can be blocked by interfering with the calcium-dependent and the calcium-independent pools of dopamine during conditioning.

1.6.2. LATENT INHIBITION OF CLASSICAL CONDITIONING

When animals are repeatedly exposed to a stimulus prior to its pairing with the UCS, that stimulus becomes much less effective in eliciting a CR. This phenomenon is known as latent inhibition (Mackintosh, 1974). For example, in Pavlov's (1927) original experiments, a bell was rung approximately simultaneously with the presentation of food to dogs, and the dogs salivated. Subsequently, when the bell was presented alone, salivation was elicited. However, if prior to conditioning, the bell was rung several times in the absence of the presentation of the food, then latent inhibition increased the number of CS-UCS pairings required before the bell elicited salivation, and also decreased the number of presentations of the bell (the CS) in the absence of food that was required to extinguish salivation to the CS once conditioning had occurred.

1.6.3. CLASSICAL CONDITIONING OF MOTOR STIMULANT EFFECTS

An animal model for studying stimulant addiction may be created by measuring the effect of psychomotor stimulants on horizontal (locomotor) and vertical (rearing) activity (Beninger and Hahn, 1983; Beninger and Herz, 1986; Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1991,1992a,b). Since classically conditioned effects in humans induce "drug craving", and the mesolimbic/mesocortical areas in the brain

provide the substrate for both stimulant-induced locomotion and for reinforcement, stimulant-conditioned locomotion in animals may serve as a model for the classical conditioning of effects that lead to the "drug craving" (Beninger and Hahn, 1983; Martin-Iverson and McManus, 1990).

The study of stimulant-conditioning can be broken down into the establishment and the expression of conditioning (Beninger and Hahn, 1983; Martin-Iverson and McManus, 1990). In an attempt to block the establishment of stimulant conditioning, animals receive a specific combination of vehicle, stimulant, and antagonist prior to the test boxes during the conditioning period, and receive only a vehicle administration on the test day to determine if establishment of conditioning was blocked (Beninger and Hahn, 1983; Beninger and Herz, 1986; Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1991,1992a,b). Since addicts have already established conditioned effects when they seek treatment, the expression of stimulant conditioning must be blocked in their case. Therefore, to block the expression of stimulant conditioning in the animal model, rats are administered with only vehicle or the stimulant drug during conditioning, and then only with vehicle and/or antagonists on the test day.

1.6.4. NEURAL SUBSTRATES OF CLASSICALLY CONDITIONED MOTOR EFFECTS

A role for dopamine in stimulant-conditioned motor behaviours is a relatively recent finding. In the first such study, amphetamine-conditioned head bobbing and sniffing were classically conditioned, and then after reconditioning, the expression of these behaviours was blocked with haloperidol (Schiff, 1982). Studies have also shown that

pimozide blocks the establishment but not the expression of amphetamine- and cocaine-conditioned locomotion and rearing (Beninger and Hahn, 1983; Beninger and Herz, 1986). Therefore, it was suggested that the activation of dopaminergic neurons produces a change in the brain that, once established, cannot be blocked by D_2 dopamine receptor blockade. Pimozide has also blocked the establishment of amphetamine-conditioned sterotypy, and the establishment and the expression of apomorphine-conditioned stereotypy (Hiroi and White, 1989). In addition, the destruction of dopaminergic terminals in the nucleus accumbens with 6-OHDA blocked the development of amphetamine-conditioned locomotion (Gold et al., 1988). However, although 6-OHDA lesions blocked amphetamine-conditioning, it is impossible to separate the establishment from the expression in this case, since dopamine would have to be replaced on the test day to do so. Therefore, it was not clear whether dopamine release in the nucleus accumbens was necessary for the establishment of amphetamine-conditioning. In another study, neither haloperidol nor the D_1 receptor antagonist SCH23390 blocked the expression of apomorphine-induced contralateral circling (Carey, 1990). In the establishment of amphetamine- or (+)-4-propyl-9-hydroxynaphthoxazine (PHNO)-conditioned locomotion in rats, the direct locomotor activating effects of the two stimulants during conditioning days, but not the conditioned locomotor effects on the test day, were blocked by dopamine D_1 and D_2 receptor antagonists (SCH23390 and haloperidol, respectively) given separately or together (Martin-Iverson and McManus, 1990). Thus, the interaction of dopamine with its receptors may not be necessary for the conditioning of amphetamine's effects to occur.

The discrepancy between the studies with D_1 or D_2 receptor antagonists administered separately or together, and the studies with pimozide, may be explained by the fact that pimozide, which was previously thought to be a relatively specific D_2 receptor antagonist, was discovered to be an equipotent antagonist of dopamine D_2 receptors and L-type Ca^{2+} channels (Cohen et al., 1986; Tecott et al., 1986; Enyeart et al., 1987). Recent work suggests involvement of L-type Ca^{2+} channels in classically conditioned motor effects of amphetamine (DiLullo and Martin-Iverson, 1991,1992a,b).

αMPT blocked amphetamine-unconditioned, but not amphetamine-conditioned locomotion (DiLullo and Martin-Iverson, 1991). To rule out conditioned effects by norepinephrine, rats were injected with N-(chloroethyl)-N-2-bromobenzylamine (DSP4), a neurotoxin specific for noradrenergic terminals. DSP4 had no effect on amphetamine-unconditioned or amphetamine-conditioned locomotion, although norepinephrine was depleted to between 1 and 14% of controls in forebrain areas, and to between 27 and 54% of controls in diencephalic areas (DiLullo and Martin-Iverson, 1991). These findings indicate that neither the Ca^{2+}-independent amphetamine-induced release of dopamine nor norepinephrine action is responsible for conditioned psychomotor effects. Reserpine alone also failed to block the conditioned locomotor effects of amphetamine, which implies that the Ca^{2+}-dependent amphetamine-induced release of dopamine is not essential either, by itself, for conditioned locomotion (DiLullo and Martin-Iverson, 1992b).

On the other hand, the combination treatment of α-MPT and reserpine blocked the development of amphetamine-conditioned locomotion, although it did not block the unconditioned effects of amphetamine on all days (DiLullo and Martin-Iverson, 1992b).

This observation suggests that the development of conditioning of amphetamine's motor stimulant effects requires dopamine release from either the newly-synthesized αMPT sensitive pool, or from the vesicular reserpine-sensitive compartment (DiLullo and Martin-Iverson, 1992b). Interference of dopamine release from both pools is necessary to block conditioning of amphetamine's motor stimulant effects. The combination treatment of haloperidol, a dopamine D_2 receptor antagonist, and nimodipine, an L-type dihydropyridine Ca^{2+} channel antagonist, resulted in a block of amphetamine-unconditioned and an attenuation of the establishment of amphetamine-conditioned locomotion (DiLullo and Martin-Iverson, 1992a).The work by DiLullo and Martin-Iverson (1991, 1992b) imply that together, the Ca^{2+}-dependent and the Ca^{2+}-independent release of dopamine may be responsible for the establishment of amphetamine-conditioned behaviours.

It is unclear whether the establishment or the expression of cocaine-conditioned motor effects involves the impulse-dependent or the impulse-independent pools of dopamine or both, since pimozide was used in the study of cocaine-conditioning (Beninger and Herz., 1986). However, previous studies suggest that cocaine-induced motor effects rely on the impulse-dependent pool of dopamine (Scheel-Kruger, 1972; Braestrup, 1977; Clemens and Fuller, 1979).

1.6.5. CONDITIONED REWARD-LIKE EFFECTS OF PSYCHOMOTOR STIMULANTS

An animal model utilized to study the conditioned reinforcing effects of psychomotor stimulants is the conditioned place preferences (CPP) procedure (Swerdlow

et al., 1989). CPP has been used to study positively or negatively reinforcing properties of many classes of drugs, including stimulants, opiate and non-opiate neuropeptides, hypnotics, alcohol, and other reinforcers such as food, sucrose, saccharin, environmental novelty, and darkness (Swerdlow et al., 1989).

Training schedules for the CPP procedure can be loosely grouped into biased or unbiased. In the biased schedule, the animal's environment of initial preference is determined first, and then the drug administration is paired with the least favoured environment several times. On the test day, if animals spend more time in the least preferred environment compared to baseline, a UCS is interpreted to have reinforcing properties. However, it is not clear whether the learning process involved in overcoming an aversion is equivalent to that involved in forming a preference; i.e., drugs that increase a preference for an initially least preferred environment may not increase, or may actually decrease, the preference for an initially preferred environment (Schenk et al., 1985). Familiarity is important in environmental preference (Carr et al., 1989), and the animal gains exposure to only the initially least preferred environment in the biased paradigm.

In the unbiased training schedule, animals are trained with one of two distinct treatments, drug on one day in one environment (the CS+), and vehicle on another day in another environment (the CS-) (Martin-Iverson et al., 1985). The number of animals receiving drug in each environment is counterbalanced, so that equal numbers in each group receive the UCS in each environment. On the test day for CPP, animals are given free access to the training environments. Sometimes an habituation period is included prior to training, so that a baseline can be established for comparison to the test day.

There are roughly 3 stages in the formation of a CPP (Swerdlow et al., 1989); 1) a UCS produces an affective change in an animal, 2) the animal associates affective change with a distinct environment, and 3) the animal recalls the association in the absence of the UCS but in the presence of the CS+. These 3 components can be manipulated separately. For example, sucrose has both reinforcing and memory enhancing effects in rats whereas saccharin is reinforcing but appears to have a much smaller mnemonic component. It has been suggested that mnemonic effects derive from post-ingestional outcomes while reinforcing effects derive from taste (Messier and White, 1984). Sucrose resulted in CPP but saccharin did not, although rats preferred a saccharin-paired environment if they received non-contingent post-training treatments with the memory enhancers glucose or amphetamine (White and Carr, 1985). However, saccharin has powerful reinforcing properties as determined by simple consumption, preference tests, and conditioned taste preference/aversion paradigms (Young and Madsen, 1963; Messier and White, 1984). These results suggest that CPP may reflect processes other than reinforcement. Thus, care must be taken in interpreting CPP.

1.6.6. NEURAL SUBSTRATES OF CONDITIONED REWARD-LIKE EFFECTS OF PSYCHOMOTOR STIMULANTS

CPP and self-administration has been observed in animals with psychomotor stimulants such as (+)-amphetamine (Spyraki et al., 1982b; Swerdlow and Koob, 1984), cocaine (Spyraki et al. 1982a; Pani et al., 1991a), methylphenidate (Martin-Iverson et al., 1985), the direct dopamine agonist apomorphine (van der Kooy et al., 1983), the endogenous trace amine β-phenylethylamine (Gilbert and Cooper, 1983), nicotine (Fudala

et al., 1985), the clinical antidepressants bupropion (Ortmann, 1985) and nomifensine (Martin-Iverson et al., 1985), and others.

The role of dopamine in CPP produced by stimulants has not been as clear as for IVSA. Amphetamine-CPP is blocked by pretreatment with the D_2 dopamine receptor antagonist haloperidol, and by depletion of nucleus accumbens dopamine by 6-OHDA (Spyraki et al., 1982b). CPP resulted when amphetamine was injected into the nucleus accumbens, but not when injected more dorsally into the caudate nucleus (Carr and White, 1983). Denervation of nucleus accumbens dopamine resulted in supersensitive CPP in response to D_1/D_2 agonist apomorphine (Staunton et al., 1982; van der Kooy et al., 1983), and following 6-OHDA depletion of dopamine in nucleus accumbens, apomorphine injected into the nucleus accumbens induced CPP and locomotor activity at 1/10 the normal dose (van der Kooy et al., 1983).

Cocaine-CPP was not blocked by pretreatment with haloperidol, pimozide, depletion of nucleus accumbens dopamine by 6-OHDA (Spyraki et al., 1982c), or by the D_1/D_2 dopamine receptor antagonist α-flupenthixol (Mackey and van der Kooy, 1985), although cocaine-CPP induced by intracerebroventricularly administered cocaine was blocked by pimozide (Morency and Beninger, 1986). Similarly, methylphenidate-CPP was not blocked by haloperidol or by 6-OHDA-induced depletions of central dopamine (Martin-Iverson et al., 1985).

In addition to dopamine D_2 receptors, D_1 receptors are probably also involved in CPP. Conditioned place aversion resulted when the D_1 agonist SKF38393 was injected intra-peritoneally (Hoffman and Beninger, 1989; White et al., 1991), but CPP resulted

following injection of SKF38393 into the nucleus accumbens (White et al., 1991). The D_1 antagonist SCH23390, blocked morphine- (Leone and DiChiarra, 1987), and amphetamine-CPP (Leone and DiChiarra, 1987; Hoffman and Beninger, 1989), and CPP produced by pipradrol (White and Hiroi, 1992), which, like cocaine, blocks dopamine uptake. Finally, the mixed D_1/D_2 antagonist, α-flupenthixol, blocked the establishment of amphetamine- (Mackey and van der Kooy, 1985) and cocaine-induced (Aulis and Hoebel, 1984) place preference conditioning in rats.

Cocaine-CPP was blocked by the dihydropyridine L-type Ca^{2+} channel antagonist isradipine (Pani et al., 1991a). Therefore, the relationship between L-type Ca^{2+} channels and cocaine's effects should be examined.

1.6.7. L-TYPE Ca^{2+} CHANNELS

The role of L-type Ca^{2+} channels in neurons is not clear. These voltage-sensitive Ca^{2+} channels are located in cells such as skeletal, cardiac, and smooth muscle, in most types of neurons, excitable endocrine cells, fibroblasts, and in two types of astrocytes (Miller and Fox, 1990). Changes in membrane potential can alter the potency of the dihydropyridines over 3 orders of magnitude, and their greatest efficacy occurs at the most depolarized potentials (Bean, 1984; Sanguinetti and Kass, 1984). The L-type Ca^{2+} channels that have been found on some nerve terminals do not appear to be directly involved in regulation of K^+-evoked neurotransmitter release (Massieu and Tapia, 1988), but do appear to be indirectly involved through Ca^{2+}-dependent modulation of K^+-evoked neurotransmitter release via second messengers (Tsien et al., 1988; Gandhi and Jones, 1992; Bielefeldt and Jackson, 1993).

Two dihydropyridines, nimodipine and isradipine, have been shown to block increases in extracellular dopamine and motor activity observed after cocaine treatment (Pani et al., 1990a,b,1991a,b), and nimodipine blocked an increase in D_1 and D_2 receptor occupation by cocaine (Burger and Martin-Iverson, 1994). Nimodipine has also blocked the discriminative stimulus properties of cocaine (Callahan and Cunningham, 1990) and amphetamine (Nencini and Woolverton, 1988), and catecholamine synthesis induced by cocaine (Pileblad and Carlsson, 1987). Blockade of dopamine release by dihydropyridines may be via cell body dendritic L-type Ca^{2+} channels that regulate the excitability of dopamine neurons, since action potentials appear to be necessary for cocaine's effects (Shore, 1976; Ross, 1977; Hyttel, 1978). Therefore, dopamine may play an important role in both amphetamine- and cocaine-conditioned reward, but the observation of different effects on amphetamine- or cocaine-CPP by drugs affecting dopamine neurotransmission may have resulted from differing mechanisms of action of the two stimulants.

In addition to the dihydropyridines, there are other types of calcium channel antagonists. Three of these are the phenylalkylamines, such as verapamil and ludopamil, and the benzothiazepines, such as diltiazem (Miller and Fox, 1990), and the diphenylalkylamines, such as flunarizine (Pani et al., 1990a). Each class of antagonist preferentially binds at a specific site on the L-type Ca^{2+} channel (Kokubun et al., 1986; Hosey and Lazdunski, 1988). Phenylalkylamines and benzothiazepines are effective at blocking some cardiac arrhythmias, since these are cases where L-type Ca^{2+} channels are opening and closing at a high frequency (Kass and Krafte, 1987). On the other hand, in addition to their effects on stimulant-induced dopamine activation and behaviours, the

dihydropyridines are more effective in the treatment of hypertension and angina, since membrane potential is the important factor here (Miller and Fox, 1990).

The phenylalkylamine and benzothiazepine L-type Ca^{2+} channel antagonists may be less effective at blocking the effects of psychomotor stimulants than are the dihydropyridines (Grebb, 1986; Pani et al., 1990a), and did not block cocaine-induced increases in extracellular dopamine concentration (Pani et al., 1990a, 1991b). However, verapamil blocked amphetamine-induced circling in 6-OHDA lesioned rats, amphetamine-induced catecholamine synthesis *in vitro* (Uretsky et al., 1979) and amphetamine-CPP (Pucilowski et al., 1993). The diphenylalkylamine, flunarizine (20 mg/kg) potentiated cocaine-induced dopamine overflow and motor activity (Pani et al., 1990a,c, 1991b), but did not affect amphetamine-induced activity until a higher dose (50 mg/kg) was used, which blocked amphetamine-induced motor activity (Grebb, 1986). Similarly, the dihydropyridine, nifedipine, blocked amphetamine-induced motor activity, although verapamil and diltiazem had no effect (Grebb, 1986). Therefore, it appears that the dihydropyridines may be the most important Ca^{2+} channel antagonists for the blockade of cocaine-induced effects, although the results with amphetamine are not as clear.

1.7. THESIS OBJECTIVES

This thesis was initially concerned with determining the role of dopamine and L-type Ca^{2+} channels in 1) the establishment and the expression of cocaine-conditioned motor effects, 2) in the conditioned reinforcing effects of cocaine, and in 3) cocaine-induced behavioural sensitization to cocaine's motor stimulant effects. During the pursuit of this

goal, evidence against classical conditioning as an explanatory concept for context-specific effects of cocaine was obtained. Therefore, a second goal was to determine if the effect of cocaine on locomotion (horizontal activity) and rearing (vertical activity) could be adequately explained by classical conditioning.

CHAPTER 2. NIMODIPINE AND HALOPERIDOL ATTENUATE BEHAVIOURAL SENSITIZATION TO COCAINE BUT ONLY NIMODIPINE BLOCKS THE ESTABLISHMENT OF CONDITIONED LOCOMOTION INDUCED BY COCAINE[1]

2.1. INTRODUCTION

The establishment of classical conditioning of the locomotor effects of amphetamine and cocaine has been shown to be blocked by pimozide (Beninger and Hahn, 1983; Beninger and Herz, 1986). Pimozide blocks both dopamine D_2 receptors and L-type calcium channels, with approximately equal potency. The establishment of the classical conditioning of amphetamine's locomotor effects are not blocked by haloperidol, a relatively selective antagonist for D_2 receptors that does not have appreciable action on L-type calcium channels (Martin-Iverson and McManus, 1990). In addition, an L-type calcium channel antagonist, nimodipine, also failed to block the establishment of the conditioning of amphetamine-induced locomotion, but administration of haloperidol and nimodipine together to rats does mimic the effect of pimozide on blocking the establishment of conditioning (DiLullo and Martin-Iverson, 1992b). Therefore, the classical conditioning of amphetamine's effects appear to be dependent upon at least two separate processes, one which is neuroleptic sensitive and one which involves the impulse-dependent L-type calcium channels. Other work (DiLullo and Martin-Iverson 1991, 1992a) has shown that the conditioning of amphetamine's locomotor effects does indeed involve

1 A version of this chapter has been published in *Psychopharmacology*, **113**:404-410 (1994).

two separate processes: Ca^{++}-dependent release of dopamine from vesicles (reserpine-sensitive), and Ca^{++}-independent release from a newly-synthesized dopamine compartment (sensitive to synthesis inhibition by α-MPT). However, the mechanisms underlying the conditioning of cocaine have not been clarified to the same extent.

The purposes of the present experiments were to establish dose-response relationships for the classical conditioning of cocaine's locomotor effects and the development of locomotor sensitization to cocaine, and to determine the role of Ca^{2+} channels and D_2 receptors in the development of these two processes. Rats received cocaine injections prior to confinement in a unique environment or in their home cages as a pseudo-conditioned control for context-independent effects. The effects of haloperidol, a dopamine antagonist relatively selective for D_2-like receptors, and nimodipine, an L-type calcium channel blocker, on the establishment of cocaine conditioning and sensitization were also investigated. These two drugs were chosen because they have previously been shown to block the conditioning of amphetamine's locomotor effects when given in combination but not when given alone (DiLullo and Martin-Iverson, 1992b).

2.2. MATERIALS AND METHODS

2.2.1. SUBJECTS

In both experiments, experimentally naive male Sprague-Dawley rats weighing between 250 and 350 g were purchased from the Health Sciences Animal Services of the University of Alberta. All rats were housed in pairs in a climatically controlled room (20-22°, humidity=50%). They were on a 12 hour light-dark cycle (0700 to 1900) with free

access to food and water. All procedures used were approved by the Health Sciences Animal Care Committee as following Canadian Council of Animal Care (CCAC) recommendations for animal use in research.

2.2.2 DRUGS

Nimodipine, provided courtesy of Dr. A. Scriabine (Miles Institute for Preclinical Pharmacology, Miles Inc.), was dissolved in a solution of polyethylene glycol 400 to a final concentration of 10 mg/ml. Haloperidol was purchased from McNeil Pharmaceutical in 1 ml ampoules containing 5 mg haloperidol dissolved in a solution of methylparaben (1.8 mg), propylparaben (0.2 mg), and lactic acid. This solution was further diluted to a final concentration of 0.05 mg/ml haloperidol with double-distilled water. Cocaine hydrochloride, purchased from British Drug Houses, was prepared in 5, 10, or 20 mg/ml solutions using double-distilled water.

2.2.3. APPARATUS

The locomotor activity test boxes measured 25 cm (H) x 25 (W) x 30 (L) and contained two infrared photocell assemblies placed 3 cm from the floor and 14 cm apart, equidistant from the end walls. By adjusting the time-constant of the response circuit, the sensitivity of the photocells was set so that only gross movements were counted. Fine repetitive movements of the head, tail, and paws were excluded.

2.2.4. PROCEDURE

Rats in all groups were habituated to their home cages for 7 days prior to the experiment. Both experiments included daily injections of cocaine for ten consecutive days with a 60 min measurement of locomotor activity on each of these days, followed by 3

days in which the rats were left in their home cages, with a test given on Day 14 when all animals received vehicle injections prior to locomotor testing for 60 min. Half of the rats received the drugs paired with the test boxes (Paired), and the other half received the drugs 2 h after removal from the test boxes while the animals were in their home cages (Unpaired). The latter groups served as the "pseudo-conditioning controls". In the first experiment, the test day was followed by an additional test on day 15, in which all animals received a challenge dose of cocaine-HCl (10 mg/kg, IP) prior to measurement of locomotor activity.

Groups of rats in experiment 1 were given cocaine (0 [vehicle], 5, 10, or 20 mg/kg, IP), with 12 rats per group for a total of 96 animals. The drug groups in experiment 2 were VVV, VVC, VNV, VNC, HVV, HVC, HNV, and HNC where V = vehicle, H = haloperidol, N = nimodipine, C = cocaine. Each group included 12 animals for a total of 192 rats. Nimodipine (10 mg/kg, SC) and haloperidol (0.05 mg/kg, IP) were injected 70 minutes prior to cocaine (10 mg/kg, IP), and cocaine was injected just prior to placement of the animals in locomotor activity measuring boxes or 2 h after removal from the test boxes, while the rats were back in their home cages.

2.2.5. STATISTICS

The raw locomotor counts from each group were expressed as percent of the mean of the vehicle control for that group. The data were subjected to analysis of variance (ANOVA). Experiment 1 had two independent factors: Context (2 levels: Paired or Unpaired) and Cocaine dose (4 levels: 0 [vehicle], 5, 10, or 20 mg/kg). There was also a repeated factor for the conditioning phase of the experiment (Days with 10 levels). In

experiment 2, there were 4 independent factors: Context (2 levels: Paired or Unpaired), Cocaine (2 levels: 0 or 10 mg/kg), Haloperidol (2 levels: 0 or 0.05 mg/kg), and Nimodipine (2 levels: 0 or 10 mg/kg). There was also a repeated factor for the conditioning phase, days (10 levels). Since ANOVA with more than 2 repeated measures is unreliable due to lack of homogeneity of covariances (Vitaliano, 1982), a variety of multivariate tests of significance (Pillais Trace, Hotellings T, Wilks Lambda, and Roys F-test) were also conducted for terms involving this factor, as is standard procedure with the statistical software used (Statistical Package for the Social Sciences). Significant ANOVA results are reported in this paper only when verified by these additional tests. Significant main effects and interactions were followed by individual comparisons by the F-test for multiple comparisons (Kiess, 1989). The critical level of significance was set at $p < 0.05$.

2.3. RESULTS

2.3.1. EXPERIMENT 1

Since we were examining behavioural sensitization in CHAPTER 2, and sensitization was not evident when locomotion was examined over days, but only when considered with respect to vehicle groups, for the figures in this chapter the data for the drug groups are expressed as a percent of control groups.

Statistical analyses were conducted separately for the three phases of this experiment: the 10 days of conditioning, the drug-free test day (Day 14) after the 3 day wash-out period and the cocaine challenge day (Day 15). There were no significant

differences in the photobeam interruptions (locomotion) between the paired and unpaired groups treated with vehicle (data not shown) during conditioning. As can be seen in Figure 2, the 10 and 20 mg/kg doses of cocaine increased locomotion, and this effect exhibited a gradual augmentation over the days of treatment (behavioural sensitization). The lowest dose (5 mg/kg) given to the paired groups did not produce much locomotion nor did much evidence of sensitization emerge, except for a significant increase in locomotion on Day 10. ANOVA revealed a significant Context by Dose by Days interaction [$F(27,792) = 4.25$, p 0.001] for the conditioning phase of the experiment. On the drug-free test for conditioned locomotion on day 14, ANOVA indicated that there was a significant Context by Dose interaction [$F(3,88) = 6.78$, $p < 0.001$]. Paired groups previously treated with 10 or 20 mg/kg cocaine exhibited significantly higher levels of locomotion than did controls (Figure 3). The group of rats given 5 mg/kg cocaine paired with the testing context, and all of the unpaired controls did not exhibit increased locomotion. There was no significant difference in photobeam interruptions between the paired and unpaired vehicle groups on Day 14 (unpaired mean=209, SEM=14.6; paired mean=188.8, SEM=21.9]. ANOVA also indicated that there was a significant Context by Dose interaction [$F(3,88) = 2.72$, $p < 0.05$] for the locomotor activity induced by the cocaine challenge on Day 15. The test group that received 10 mg/kg cocaine paired with the test context during conditioning had the most robust sensitization in response to a challenge dose of cocaine on Day 15 (Figure 4). The group receiving 5 mg/kg during conditioning and the unpaired drug groups did not exhibit sensitization to the 10 mg/kg challenge dose of cocaine. There were no significant differences in photobeam interruptions between VEH

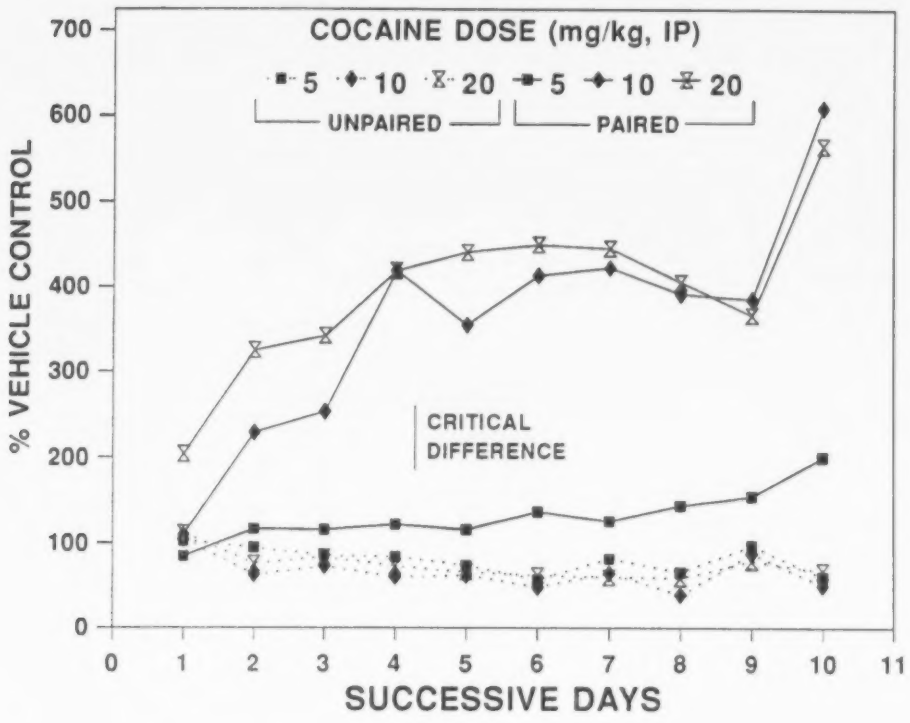

Figure 2. Motor stimulant effects of daily injections of cocaine at the indicated doses, as a percent of the locomotion of the respective vehicle groups. Injections were given to rats either immediately preceding locomotor testing (PAIRED) or 2 h after removal from the test boxes (UNPAIRED). The line representing the critical difference required for differences in the means to be significant with alpha = 0.05 is derived from the Multiple F procedure for individual comparisons. The groups receiving 10 and 20 mg/kg cocaine paired with the test boxes exhibit equivalent degrees of sensitization, and even the lowest dose develops some significant stimulant effects by Day 10 (n=12).

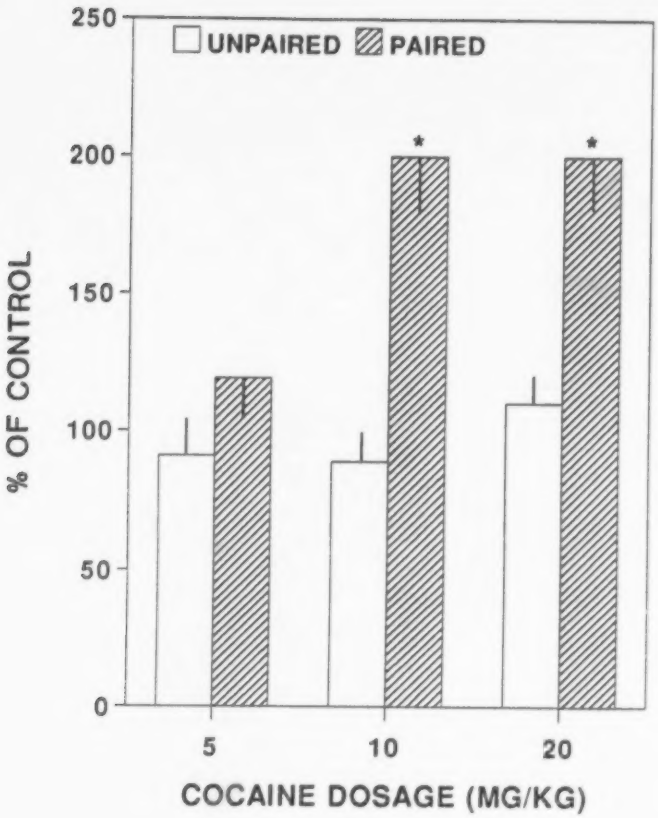

Figure 3. Motor stimulant effects conditioned to the test context by prior daily injections of cocaine at the indicated doses paired or unpaired with the test context, as a percent of the locomotion of the respective vehicle groups (data in text). Bars represent the SEM of each group. On this test, all rats were given vehicle injections only. The groups given previous injections of 10 or 20 mg/kg cocaine paired with the test context exhibited increases in locomotion relative to vehicle groups. * Significantly different from the paired vehicle injected group, $p < 0.05$ ($n=12$).

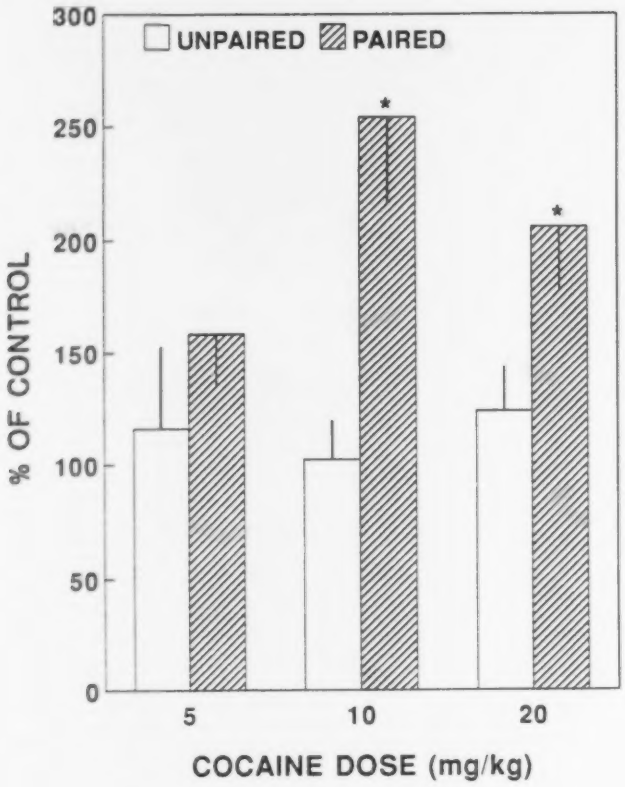

Figure 4. Motor stimulant effects of a single treatment with cocaine (10 mg/kg, IP) given to rats with a prior history of cocaine treatments at the indicated doses paired or unpaired with the test context, as a percent of the locomotion of the respective vehicle groups after a similar cocaine injection (data in text). Bars represent the SEM of each group. Only the groups given previous injections of 10 or 20 mg/kg cocaine paired with the test context exhibited sensitization. * Significantly different from the paired chronic vehicle, acute cocaine (10 mg/kg) injected group, $p < 0.05$ (n=12).

groups after the cocaine challenge on Day 15 (unpaired mean = 346.2, SEM = 87.2; paired mean = 200.8, SEM = 38.8). These results demonstrate that cocaine sensitization was context-dependent, i.e., it was absent in the cocaine-treated unpaired groups.

2.3.2. EXPERIMENT 2

In this experiment, statistical analysis was conducted separately for the 10 days of conditioning (and drug treatments) and the drug-free test day. All groups showed a relatively high level of locomotor activity on Day 1 which decreased substantially thereafter (habituation). No significant differences were observed in the locomotor activity among the groups that received vehicle or haloperidol, vehicle or nimodipine and vehicle control groups (ie. VVV, HVV, VNV, HNV) either with injections unpaired with the test boxes or with injections paired with exposure to the test boxes during conditioning. Groups receiving cocaine 2 h after removal from the test boxes (unpaired) did not exhibit locomotor activity in the test boxes 22 h after cocaine treatments different from their respective vehicle controls (Figure 5). On the other hand, rats receiving cocaine paired with the test boxes exhibited levels of locomotion between 115-210% of controls on Day 1, and between 275-480% by Day 10 (Figure 6). These data demonstrate the development of context-specific cocaine-induced behavioural sensitization to 10 mg/kg cocaine. This sensitization was attenuated (but not blocked) similarly by nimodipine and haloperidol. When the two antagonists were both given to the rats, the degree of attenuation was decreased. The validity of these observations are supported by a significant Context by Haloperidol by Nimodipine by Cocaine by Days interaction in the locomotor behaviour during conditioning [$F(9,1584)=2.19$, $p < 0.025$]. On the drug-free Day 14 testing for

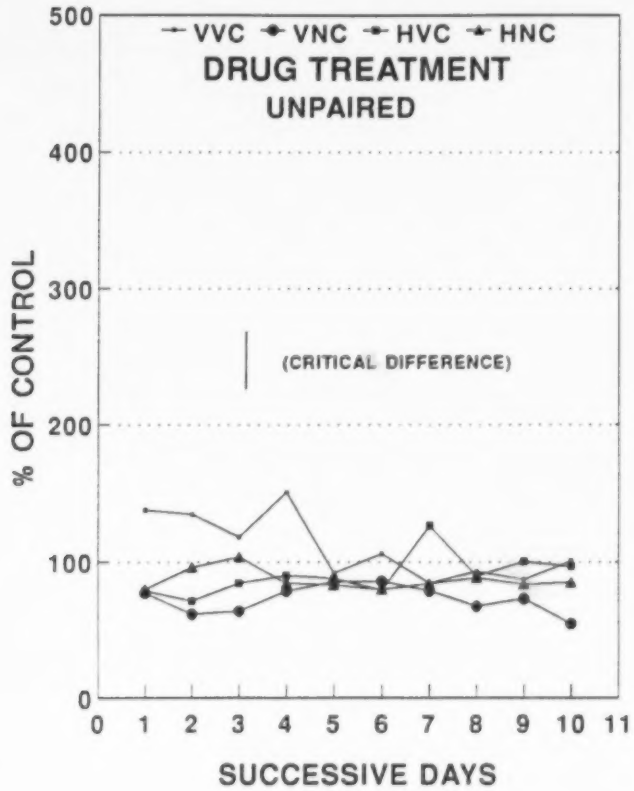

Figure 5. Motor stimulant effects over 10 days of vehicle (V), 0.05 mg/kg haloperidol (H), 10 mg/kg nimodipine (N) and 10 mg/kg cocaine (C) unpaired with the test context (injections 2 h after locomotor testing), as a percent of the respective vehicle injections (ie. VVV-, VNV-, HVV- or HNV- treated groups). For example, the data from the VVC (vehicle + vehicle + cocaine) group are expressed as a percent of the mean from the VVV group, and the HNC (haloperidol + nimodipine + cocaine) group is expressed as a percent of the mean of the HNV group. Note that there are no significant differences among the groups. The critical difference line was obtained using the Multiple F test for individual differences (n=12).

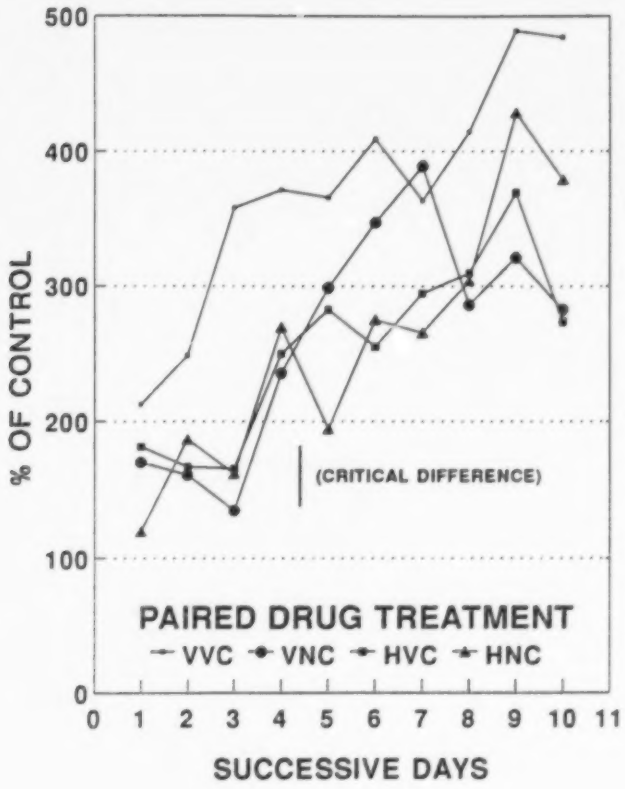

Figure 6. Motor stimulant effects over 10 days of vehicle (V), 10 mg/kg nimodipine (N), 0.05 mg/kg haloperidol (H) and 10 mg/kg cocaine (C) paired with the test context, as a percent of the respective vehicle (ie. VVV, VNV, HVV and HNV). For example, the data from the VVC (vehicle + vehicle + cocaine) group are expressed as a percent of the mean from the VVV group, and the HNC (haloperidol + nimodipine + cocaine) group is expressed as a percent of the mean of the HNV group. Note that all groups develop some degree of sensitization, but that co-treatment with either haloperidol or nimodipine attenuate sensitization. The CD line was obtained using the Multiple F test for individual differences (n=12).

the presence of conditioned locomotion, ANOVA demonstrated a significant Context by Nimodipine by Cocaine interaction [$F(1,176)=4.35$, $p < 0.038$]. Pretreatment of rats with haloperidol during the establishment of conditioning was without significant effects on the drug-free test of conditioned locomotion. There were no significant differences between pairs of the appropriate vehicle controls (Figure 7). Context-specific cocaine-conditioning was elicited and this conditioning was completely blocked by previous treatments during conditioning with nimodipine and by the combination of nimodipine and haloperidol but not by pretreatment during conditioning with haloperidol alone (Figure 8).

2.4. DISCUSSION

The present results indicate that when repeated daily injections of cocaine (5 - 20 mg/kg, IP) to rats are paired with a unique environment, the locomotor stimulant effects are gradually augmented over 10 days (behavioural sensitization), as has been previously reported (Barr et al., 1983; Beninger and Herz, 1986). In experiment 1 of this study, sensitization was not apparent in rats that had received similar cocaine treatments in a context different from the locomotor testing when challenged with cocaine in the test context. Accompanying context-specific sensitization was the classical conditioning of the locomotor stimulant effects of cocaine to contextual stimuli. Only the groups that displayed a significant conditioned effect in experiment 1 also exhibited sensitization after a challenge dose of 10 mg/kg cocaine. Sensitization to cocaine has previously been found to be context-specific (Post et al., 1981; Weiss et al., 1989).

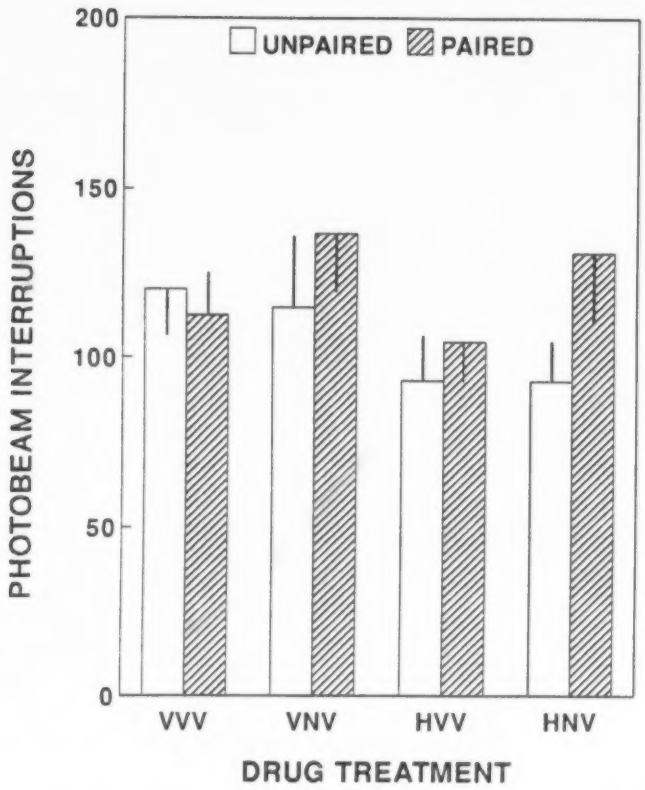

Figure 7. Motor stimulant effects (mean number of photobeam interruptions + or - SEM) conditioned to the test context by prior daily injections of vehicle (V), or 0.05 mg/kg haloperidol(H), 10 mg/kg nimodipine (N) or V, and V paired or unpaired with the test context. On this test, all rats were given vehicle injections only. Note that there were no significant differences among the groups (n=12).

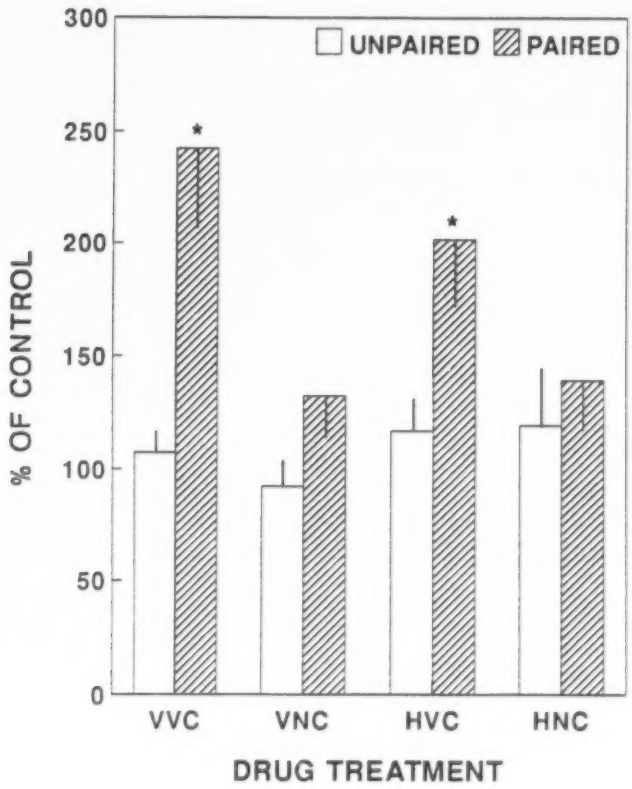

Figure 8. Motor stimulant effects (% of appropriate vehicle controls as shown in Fig. 6, + or - SEM) conditioned to the test context by prior daily injections of vehicle (V), or 0.05 mg/kg haloperidol(H), 10 mg/kg nimodipine (N) or V, and 10 mg/kg cocaine paired or unpaired with the test context. On this test, all rats were given vehicle injections only. Note that only nimodipine blocked the conditioned locomotion produced by cocaine. Significantly different from the appropriate control group, and from the unpaired group with the same drug treatment, $p < 0.05$ (n=12).

Although the expression of behavioural sensitization to cocaine in experiment 1 appeared to be completely due to classical conditioning when rats were treated only with cocaine, additional pharmacological treatments showed that behavioural sensitization may not be explained by classical conditioning alone. In experiment 2, haloperidol, a relatively selective antagonist for dopamine D_2 receptors, attenuated the development of sensitization during conditioning days without influencing classical conditioning. The ability of haloperidol to decrease sensitization to cocaine (Weiss et al., 1989) and apomorphine (Mattingly and Rowlett, 1989) has been published previously. Failure of haloperidol to block the establishment of amphetamine-conditioned locomotion has also been reported (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1992b). Also in experiment 2, nimodipine only partially blocked the development of sensitization during conditioning days, but completely blocked classical conditioning of cocaine's locomotor effects to contextual stimuli. This observation suggests that the previously found blockade of the establishment of cocaine-conditioned locomotion by pimozide (Beninger and Herz, 1986) was due to the L-type calcium channel blocking actions of pimozide, not to its ability to antagonize dopamine D_2 receptors. The present results support evidence that sensitization and conditioning to cocaine may be dissociated.

This apparent "double-dissociation" may weaken explanations of the effects due to differential sensitivity of the conditioning and sensitization test procedures to blockade. The test for the presence of conditioning is likely more susceptible to blockade than is the test for sensitization because of the absence of drug-related cues (possibly peripheral effects such as increases in heart rate), and the fact that the conditioning test occurs during

extinction, while sensitization is tested during an additional training trial.

The most parsimonious explanation therefore appears to be that behavioural sensitization and conditioning may develop from separable processes. Sensitization appears to be controlled by conditioning factors, but a non-associative sensitization process is also present, and can be unmasked under special circumstances. One cautionary note concerning this conclusion should be observed: the effects of nimodipine and haloperidol at attenuating sensitization were not additive, as would be expected if the drugs acted by blocking independent processes (except on the first day of the conditioning period (figure 5, but also see figure 8 in CHAPTER 3)).

In the present study, sensitization in experiment 1 was found to be context-specific, but the results from experiment 2 suggest that sensitization may be doubly dissociated from classical conditioning by haloperidol, which attenuated the development of sensitization during conditioning days without influencing conditioning, and by nimodipine, which completely blocked conditioning but only partially attenuated the development of sensitization during conditioning days. However, although the data support the claim that sensitization and conditioning of cocaine-induced locomotor activity may develop from separate processes, an additional control may have been helpful. Future studies should include a cocaine challenge injection administered to all groups in the absence of nimodipine and/or haloperidol, to determine if the actual establishment of cocaine-induced sensitization can be blocked by nimodipine and/or haloperidol.

Similar to our results with cocaine, a dissociation between sensitization and conditioning has been reported for amphetamine, using behavioural procedures (Stewart

and Vezina, 1991). Amphetamine-induced sensitization of locomotion and rearing were found to be completely context-dependent. However, if the conditioning component underwent extinction over a number of trials, a degree of context-independent sensitization emerged for locomotion but not for rearing. Thus, at least one type of behaviour exhibited some degree of non-associative sensitization when classical conditioning was extinguished. Stewart and Vezina suggested that the masking of the relatively weak non-associative sensitization by classical conditioning could have occurred by the association of the testing context of the pseudo-conditioned control groups with the absence of drug, making the test context a CS- for (in their case) amphetamine. After extinction, the CS- properties of the test context would be extinguished, and the non-associative sensitization emerges. In this way, classical conditioning procedures come to control the development and expression of sensitization to stimulants, over-riding a non-associative component.

It has been shown that continuous administration of amphetamine (Martin-Iverson, 1991; Nielsen, 1981), cocaine (Post et al., 1981) and a direct agonist for D_2 and D_3 dopamine receptors, PHNO, Martin-Iverson et al., 1987, 1988a, 1988b; Martin-Iverson, 1991) result in behavioural tolerance during the day. Thus, a treatment regimen that does not allow specific contextual stimuli to become associated with the drug effect does not appear to produce sensitization. The same treatment regimens that produce behavioural tolerance during the day also result in sensitization at night (Martin-Iverson, 1991; Martin-Iverson et al., 1987, 1988a, 1988b). These effects cannot be explained by associative processes, but may be due to circadian rhythms since the pattern of tolerance/sensitization follows the free-running rhythms in motor activity under conditions of constant lighting

(Martin-Iverson and Yamada, 1992). However, recent work from our laboratory has shown that the same is not true for cocaine: tolerance occurs to cocaine-induced behaviours during both day and night (unpublished data).

There appear to be fundamental differences in the sensitization to, and conditioning of, cocaine's motor stimulant effects relative to those of amphetamine and PHNO. Besides the differences in nocturnal sensitization just noted, neither haloperidol nor nimodipine block the establishment of conditioning of amphetamine's locomotor effects, but the two drugs given together can block this conditioning (DiLullo and Martin-Iverson, 1992b; Martin-Iverson and McManus, 1990). Haloperidol was found to be ineffective at attenuating conditioning to a direct and selective agonist for dopamine D_2 receptors (Martin-Iverson and McManus, 1990). Therefore, it appears that neither stimulant-induced sensitization nor classical conditioning of stimulant effects are functions of single common mechanisms, but can occur by a variety of mechanisms, differing with the stimulants and treatment regimens used.

Previous work has shown that the conditioning of amphetamine's behavioural effects involves two independent processes, a Ca^{2+}-dependent and a Ca^{2+} -independent mechanism, each of which can support conditioning in the absence of the other (DiLullo and Martin-Iverson, 1992a, 1992b). The present finding that an L-type calcium channel antagonist (nimodipine) can block the conditioning of cocaine's motor effects indicates that this conditioning occurs via a single Ca^{2+}-dependent mechanism.

Other research supports a role for L-type Ca^{2+} channels in cocaine-induced effects. For example, nitrendipine blocks the cardiac toxicity and lethal effects of cocaine (Trouve

and Nahas, 1986). Isradipine inhibits a cocaine-conditioned place preference (Pani et al., 1991b) and isradipine and nimodipine prevent cocaine-induced dopamine release and motor activity in rats (Pani et al., 1991a). Taken together with the present results, these data suggest the possibility that nimodipine could be effective as a treatment for cocaine addiction and for cocaine-induced psychoses.

However, since we can only interpret the implications of behavioural animal models, it is a large step from the animal model to the clinic. Another problem with the interpretation of the animal model is the dosage of drugs that were used. For example, in a 70 kg human, 30 mg is a normal dose of nimodipine (Dr. P.H. Silverstone, personal communication). On the other hand, the rats in this experiment received 10 mg/kg of nimodipine. However, the dosage of haloperidol that was used in this experiment is considered to be low for a rat (0.05 mg/kg), and would be considered a low dose in humans also (Dr. P.H. Silverstone, personal communication). Therefore, further studies will be required before a definite conclusion will be reached on the efficacy of nimodipine in the treatment of human behavioural disorders.

CHAPTER 3. EFFECTS OF NIMODIPINE AND/OR HALOPERIDOL ON THE EXPRESSION OF CONDITIONED LOCOMOTION AND SENSITIZATION TO COCAINE IN RATS[2]

3.1. INTRODUCTION

The development of conditioning of cocaine's locomotor effects are blocked by nimodipine alone, and is unaffected by haloperidol (CHAPTER 2). On the other hand, the development of sensitization to cocaine during conditioning days may be attenuated by either nimodipine or haloperidol (CHAPTER 2). In the present experiments, the effects of haloperidol and nimodipine on the expression of conditioning and sensitization to cocaine were investigated.

3.2. MATERIALS AND METHODS

3.2.1. SUBJECTS

Experimentally naive male Sprague-Dawley rats (250-350 g) were housed in pairs in a climatically controlled room (20-22 °C, humidity = 50%). They were on a 12 hour light-dark cycle (0700 to 1900) with free access to food and water.

3.2.2. DRUGS

Nimodipine, provided courtesy of Dr. A. Scriabine (Miles Institute for Preclinical Pharmacology, Miles Inc.), was dissolved in a solution of polyethylene glycol 400 to a final concentration of 10 mg/ml. Haloperidol was purchased from McNeil Pharmaceuticals in 1 ml ampoules containing 5 mg haloperidol dissolved in a solution of methylparaben

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2 A version of this chapter has been published in *Psychopharmacology*, **114**:315-320 (1994).

(1.8 mg), propylparaben (0.2 mg), and lactic acid. This solution was further diluted to a final concentration of 0.05 mg/ml haloperidol with double-distilled water. Cocaine-hydrochloride, purchased from British Drug Houses, was prepared in a 10 mg/ml solution using double-distilled water.

3.2.3. APPARATUS

The locomotor activity test boxes measure 25 cm (H) x 25 (W) x 30 (L) and contain 2 infrared photocell assemblies placed 3 cm from the floor and 14 cm apart, equidistant from the end walls. The sensitivity of the photocells is adjusted such that only gross movements are counted. Fine movements of the head, tail, and paws are excluded. Locomotor activity was measured while the animals were in the test boxes for 60 minutes on each day.

3.2.4. PROCEDURE

Rats in all groups (N = 96) were habituated to their home cages for 7 days prior to the experiment. Figure 9 shows the results of an investigation of the acute effects of haloperidol and nimodipine at blocking cocaine-induced locomotion. The results of this test are represented by the day 1 data from the conditioning period in experiment 2 of CHAPTER 2, the test involved measurement of locomotor for 60 min immediately after an injection of vehicle or cocaine (10 mg/kg, IP). Seventy min prior to cocaine treatment, the rats were given two injections, one of vehicle or haloperidol (0.05 mg/kg, IP) and one of vehicle or nimodipine (10 mg/kg, SC). The groups therefore consisted of VVV, VVC, VNV, VNC, HVV, HVC, HNV, HNC, where V = vehicle, H = haloperidol, N = nimodipine and C = cocaine.

The remainder of the experiments in CHAPTER 3 consisted of four test phases: conditioning, classical conditioning test, retraining and sensitization test. The conditioning consisted of daily injections of cocaine (10 mg/kg, IP, N = 48) or vehicle (N = 48) in a unique environment for 10 consecutive days. Immediately following the injections on each day, the rats were placed in test boxes and locomotor activity was assessed for 1 h. After the last day of conditioning, the vehicle and cocaine groups were each divided further into 4 groups matched on the basis of their locomotor activity scores by calculating the average daily level of activity of each animal over the 10 days, and then by taking the 4 rats with the highest level of activity in each of the cocaine and vehicle groups and randomly assigning them to 4 groups; the 4 rats with the next highest activity levels were then randomly assigned to one of the 4 groups, and this was continued until all rats were assigned to a specific group.

After 3 days without handling or injections to allow for drug clearance, the rats were placed in the test boxes after injections with VVV, VNV, HVV or HNV, where H = haloperidol (0.05 mg/kg, IP), N = nimodipine (10 mg/kg SC), and V = vehicle (first and third injections = IP, second injection = SC), with 24 rats in each group. Each of these groups were divided further into two groups of 12, on the basis of previous treatments (ie. vehicle or cocaine). Haloperidol and nimodipine were injected 70 minutes prior to the vehicle injections; this time interval has been established in previous experiments to be appropriate for these drugs (DiLullo and Martin-Iverson, 1992; CHAPTER 2). The rats were then re-conditioned with cocaine (N = 48) or vehicle (N = 48) for 3 days, following the same regimen as in the original conditioning. The

sensitization test was conducted identically to the classical conditioning test except that all rats received an injection of cocaine (10 mg/kg, IP) prior to placement in the boxes, instead of vehicle. Rats that were conditioned with vehicle or with cocaine were therefore injected with VVC, VNC, HVC, and HNC (N = 12 in each of 8 groups).

3.2.5 STATISTICS

The data were subjected to analysis of variance (ANOVA). In figure 9, the results were expressed as percent of the appropriate control (eg. the data from the HNC group were analysed as a percent of the HNV group); analysis of the raw data did not give substantially different results. In the conditioning phase, there was 1 independent factor (cocaine dose 2 levels: vehicle or 10 mg/kg) and 1 repeated factor (days 10 levels). In the conditioning and sensitization tests there were 3 independent factors: haloperidol (2 levels: vehicle or 0.05 mg/kg), nimodipine (2 levels: vehicle or 10 mg/kg), and previous drug treatment (2 levels: vehicle or cocaine). Locomotor activity in the retraining phase was analysed by ANOVA with 3 independent factors: previous treatment (on the conditioning test day) with vehicle or haloperidol, and vehicle or nimodipine, and current treatment with cocaine (2 levels: vehicle or 10 mg/kg), and 1 repeated factor (days 3 levels).

Since ANOVA with more than 2 repeated measures is unreliable due to lack of homogeneity of variances or covariances when there are order effects (Vitaliano, 1982), a number of multivariate tests of significance (Pillais Trace, Hotellings T, Wilks Lambda, and Roys F-test) were also conducted for terms involving this factor. Significant ANOVA results are reported in this paper only when verified by these additional tests. Significant main effects and interactions were followed by individual comparisons by the F-test for multiple comparisons (Kiess, 1989). The critical level of significance was set at $p < 0.05$.

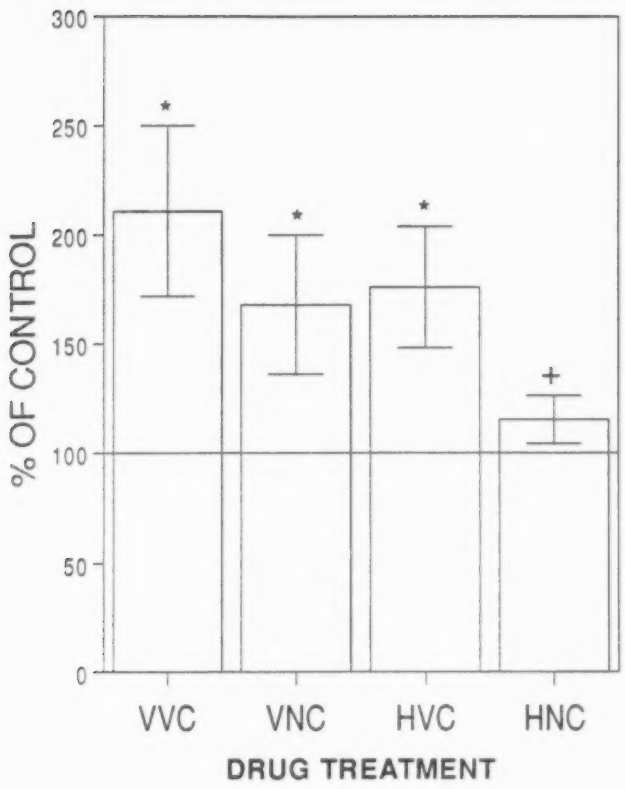

Figure 9. The effects of vehicle (V) or haloperidol (H, 0.05 mg/kg) and vehicle or nimodipine (N, 10 mg/kg) on locomotor activity induced by cocaine (C, 10 mg/kg) as measured by counting interruptions of photobeams transecting the test cages. Neither haloperidol nor nimodipine significantly decreased cocaine's effects, but the two drugs in combination blocked locomotor activity produced by cocaine. * Significantly different from controls, $p < 0.05$, Multiple F test. + Significantly different from VVC, but not from controls, $p < 0.05$, Multiple F test. (n=12)

3.3. RESULTS

Except for Figure 9, which illustrates data from day 1 of the second experiment of CHAPTER 2, the data in this section are presented as photobeam interruptions since it was apparent that sensitization was only present following ten days of a once per day injection regimen of cocaine (10 mg/kg), if data were presented as a percent of vehicle. Therefore, it was considered to be more realistic to consider the actual locomotor activity data (photobeam interruptions).

The results of the acute effects of nimodipine and haloperidol on cocaine-induced locomotion are displayed in Figure 9. Neither nimodipine nor haloperidol significantly decreased cocaine-induced locomotion at the doses employed, but the two drugs given together blocked cocaine-induced locomotion. During the 10 days of conditioning, rats given injections of vehicle exhibited a progressive decrease in activity most marked from day 1 to day 3 (Figure 10). Cocaine (10 mg/kg) increased locomotion, and this effect increased over the days of treatment relative to the vehicle group (Figure 10). ANOVA revealed a significant cocaine by days interaction ($F(9,846)=6.93$, $p < 0.001$). The expression of cocaine-conditioned locomotion was not significantly decreased by haloperidol or by nimodipine, but the combination of the two drugs attenuated the expression of conditioned locomotion, in comparison to either the group that was conditioned with cocaine but received only vehicle injections on the test day or the group that had never received cocaine but was injected with haloperidol and nimodipine on the

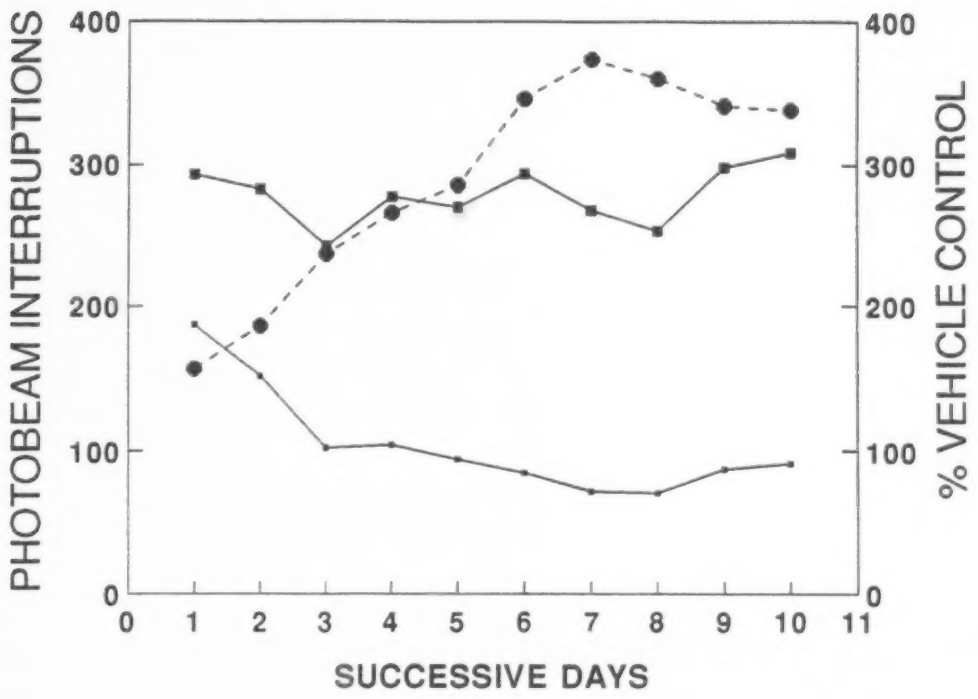

Figure 10. The effect of cocaine injections (10 mg/kg, IP) over 10 consecutive days as a percent of vehicle controls (circles and dotted lines, right axis) or as raw data (boxes with solid lines, left axis). The cocaine group differed significantly from the vehicle controls on each day (planned comparisons, Multiple F test, $p < 0.05$), and the difference between the two groups increased over the first 7 days (n=48).

test (Figure 11). ANOVA indicated that there were significant main effects of previous treatment with cocaine ($F(1,88) = 22.26$, $p < 0.001$) and of present treatment with nimodipine ($F(1,88) = 15.1$, $p < 0.001$). ANOVA indicated that only cocaine treatment had a significant effect over the 3 days of retraining ($F(1,88) = 88.0$, $p < 0.001$; data not shown). In the sensitization test, the main effects of cocaine and nimodipine were also significant, but as can be seen in Figure 12, nimodipine by itself was sufficient to attenuate cocaine-induced sensitization [Main effect of cocaine: $F(1,88) = 18.8$, $p < 0.001$; Main effect of nimodipine: $F(1,88) = 15.4$, $p < 0.001$].

3.4. DISCUSSION

The major finding of this study is that nimodipine, an L-type calcium channel antagonist, appeared to block the expression of sensitization to cocaine, but not the expression of the classical conditioning of cocaine's locomotor effects. Haloperidol, a relatively selective antagonist for dopamine D_2 receptors, appeared to be without effect on either the sensitization or the classical conditioning of cocaine's locomotor effects. However, the combination of nimodipine and haloperidol appeared to block the expression of the classical conditioning of cocaine's motor stimulant effects, similar to the results in the acute study investigating direct effects on cocaine-induced locomotion. In the acute study, neither nimodipine nor haloperidol were sufficient to block cocaine's motor stimulant effects, but the two drugs together did. However, the action of the combination treatment on cocaine sensitization was not appreciably different from the effect of nimodipine alone. These data indicate that the expression of sensitization and classical

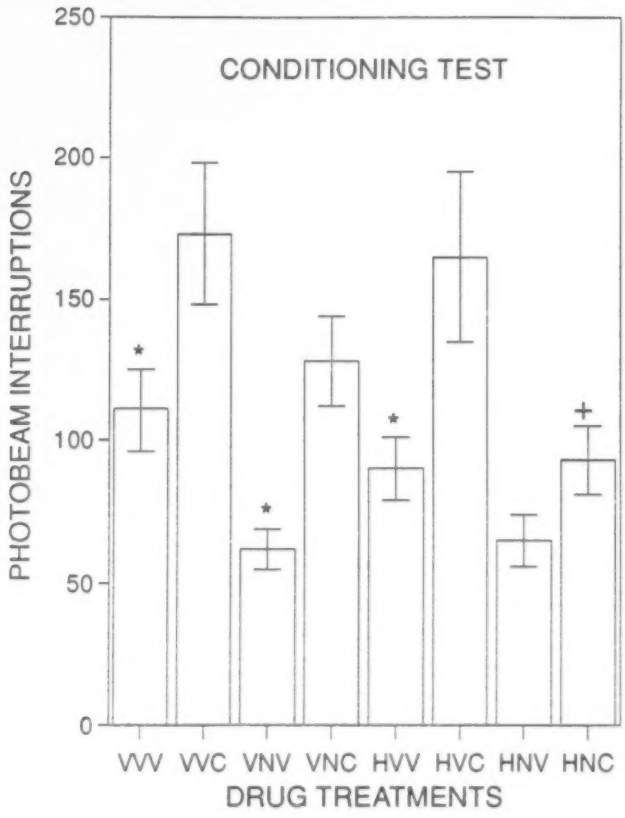

Figure 11. The effect of treatment with vehicle (V), haloperidol (H, 0.05 mg/kg, IP) or nimodipine (N, 10 mg/kg, SC) on the expression of conditioned locomotion as measured by counting photobeam interruptions (mean counts $\pm$ SEM) in an environment previously associated with cocaine treatments, but tested in the absence of cocaine treatment on this day. The final V or C in each 3-letter drug code designation refers to previous treatment history. For example, HNC refers to a group that received haloperidol and nimodipine injections 70 min prior to a vehicle injection, after which testing began, but this group had previously received 10 consecutive daily cocaine-context pairings. Note that neither nimodipine nor haloperidol alone (VNC and HVC) reduced conditioned locomotion relative to the appropriate control groups (VNV and HVV, respectively), but the combination of the two drugs (HNC) did. * Significant difference between group that received vehicle during conditioning and the comparable groups that was conditioned with cocaine, $p < 0.05$, Multiple F test. + Significantly different from VVC group, $p < 0.05$, Multiple F-test, ($n=12$).

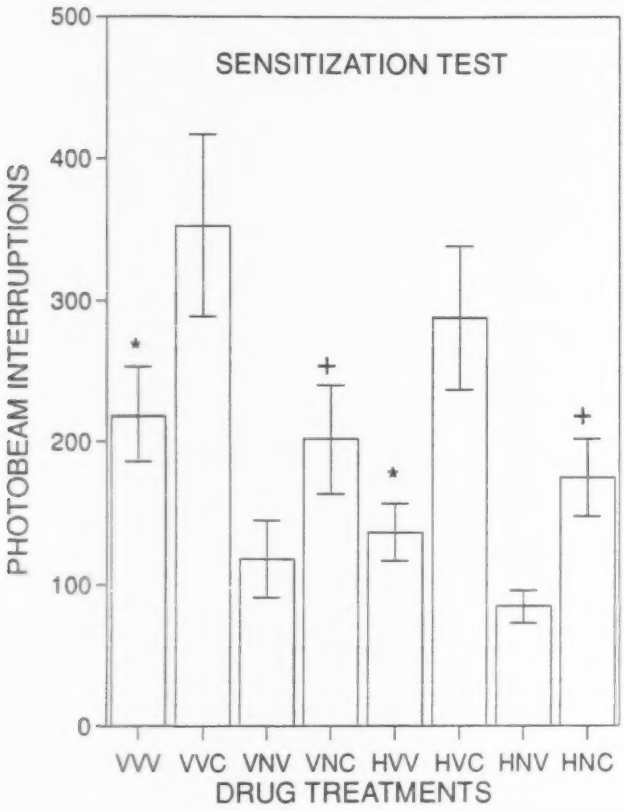

Figure 12. The effect of treatment with vehicle (V), haloperidol (H, 0.05 mg/kg, IP) or nimodipine (N, 10 mg/kg, SC) on the expression of behavioural sensitization to cocaine as measured by counting photobeam interruptions (mean counts $\pm$ SEM). The final V or C in each 3-letter drug code designation refers to previous treatment history. For example, HNV refers to a group that received haloperidol and nimodipine injections 70 min prior to a cocaine (10 mg/kg, IP) injection, after which testing began, but this group had previously received 10 consecutive daily vehicle-context pairings. Note that nimodipine (VNC and HNC) reduced cocaine sensitization relative to the appropriate control groups (VNV and HNV, respectively), but haloperidol was without additional effect. * Significant difference between group that received vehicle during conditioning and the comparable groups that was conditioned with cocaine, $p < 0.05$, Multiple F test. + Significantly different from VVC group, $p < 0.05$, Multiple F test ($n=12$).

conditioning of cocaine's locomotor effects can be pharmacologically dissociated, as was previously reported for the development of these phenomena (CHAPTER 2). It is therefore possible that there are at least some physiologically different processes underlying sensitization and classical conditioning to cocaine.

It was found in the previous study (CHAPTER 2) that nimodipine, but not haloperidol, blocked the development of classical conditioning to cocaine, and that either nimodipine or haloperidol attenuated the development of sensitization to cocaine during conditioning days. Furthermore, it was found that neither nimodipine nor haloperidol blocked the establishment of classical conditioning of amphetamine's motor stimulant effects, but the combination treatment did (DiLullo and Martin-Iverson, 1992b). The effects of these drugs on the expression of classical conditioning of cocaine are different from their effects on development of conditioning to cocaine but are similar to the development of conditioning to amphetamine: neither agent alone is effective at blocking expression, but the two antagonists given in combination do block expression of cocaine-conditioning.

Our data indicate that the effects of nimodipine and haloperidol on the expression of behavioural sensitization of locomotion to cocaine may be different from their effects on the development of sensitization to cocaine. Either of the antagonists attenuates the development of sensitization during conditioning days, but only nimodipine may block the expression of cocaine sensitization. This apparent dissociation supports the view that the neural substrates of the expression of these effects are different in some ways from the substrates of their development (Beninger and Hahn, 1983; Beninger and Herz, 1986).

However, to control for context-independent effects, this experiment should have included home cage control groups that received drug injections only in the home cages.

There has been some debate as to whether context-specific stimulant-like effects and context-specific sensitization are due to classic conditioning, or to some other process such as enhancement of the activation properties of novel stimuli (Gold et al., 1988), blockade of habituation or "behavioural reorganization" (Damianopoulos and Carey, 1992). Doubt of the applicability of Pavlovian conditioning to the observed phenomenon has arisen because the development of sensitization to cocaine is often only in relation to progressive decreases in the motor activity of the control group (i.e. chiefly due to habituation in the control group, rather than increases in the drug group). Furthermore, the level of context-elicited locomotion in the drug group on the drug-free conditioning test is often similar to the level of locomotion in the control group on the first day of testing. These characteristics are features in the present results. Figure 10 indicates that the level of locomotion induced by cocaine is relatively stable over the 10 days; sensitization is apparent only when expressed as a percent of control. In other experiments, the same dose of cocaine produces augmentation of the locomotor counts when continued over 14 days rather than 10 (Burger and Martin-Iverson, 1994). Also, sensitization is clearly not related to habituation in the control group in the locomotor activity of animals treated with a D_2 agonist, PHNO (Martin-Iverson and McManus, 1990). On the conditioning test, the experimental group in the present report exhibited higher levels of locomotion than the control group, but the level of locomotion was less than that produced by cocaine itself, and was similar to the level exhibited by the control group on the first day of testing, prior

to habituation. However, it should be remembered that the conditioning test is also a day of extinction; the level of locomotion would therefore be expected to be less. In addition, the level of locomotion on the test day can be increased by restricting the temporal association of the cues with the peak effect of the stimulant during conditioning (Hiroi and White, 1989). Finally, recent data from this laboratory have indicated that neither locomotion nor rearing behaviour exhibit patterns that can be confidently ascribed to classical conditioning, but other behaviours such as sniffing, head movements, and snout contact with a cage surface do appear to be classically conditioned to contextual stimuli (Martin-Iverson and Fawcett, in press). Classical conditioning is likely one of a variety of processes that underlie context-specific locomotion.

Interest has been growing in the conditioning of effects of stimulants in humans as a contributing factor to drug "craving" (O'Brien et al., 1988). DiLullo and Martin-Iverson (1992b) suggested that a combination treatment of nimodipine and haloperidol may be effective in attenuating this craving on the basis of the effects of the combination therapy on blocking the development of conditioning to amphetamine. However, the efficacy of nimodipine alone in blocking the establishment of cocaine conditioned locomotion (CHAPTER 2) may indicate that the addition of haloperidol is not necessary for blocking craving. The ability of isradipine, another L-type calcium channel antagonist, to block the development of place preferences induced by cocaine supports this suggestion (Pani et al., 1991). The present results indicate that the combination therapy is likely to be necessary to block craving induced by conditioned stimuli, since it is the expression of this conditioning that would require blockade in cocaine abusers. However, as discussed in

CHAPTER 2, caution must be exercised in extrapolating these results since animal models may not accurately reflect the human condition.

Certain investigators (eg. Angrist, 1983; Robinson and Becker, 1986) have suggested that behavioural sensitization to psychomotor stimulants provides an animal model of stimulant psychosis, and possibly schizophrenic psychosis. If this model has construct validity then the present results suggest that nimodipine may be an effective treatment for psychoses, since it can attenuate both the development and expression of sensitization to cocaine. Nimodipine could be an efficacious therapy or adjunct therapy for schizophrenia, since the L-type calcium channel blockers are relatively innocuous with respect to side-effects. Indeed, it has been suggested that drugs of this class can alleviate tardive dyskinesia (Bartko et al., 1991). Furthermore, serum calcium levels appear to increase during psychotic episodes (Carman and Wyatt, 1979), and preliminary uncontrolled studies have indicated a possible utility of L-type calcium channel antagonists as an adjunct therapy to neuroleptics for the alleviation of schizophrenic symptoms (Bartko et al., 1991; Lapierre, 1978), although this has been questioned (Silverstone and Grahame-Smith, 1991).

CHAPTER 4. NIMODIPINE PREVENTS THE ESTABLISHMENT OF COCAINE-CONDITIONED PLACE PREFERENCES[3]

4.1. INTRODUCTION

There is evidence that nimodipine, a dihydropyridine L-type Ca^{2+} channel antagonist, can block both cocaine-conditioning and sensitization. The effects of nimodipine on the two phenomena can be dissociated from each other. Although nimodipine alone blocked the establishment of cocaine-conditioned locomotion and attenuated the establishment of sensitization during conditioning days (CHAPTER 2), the combination of nimodipine and haloperidol was required to block the expression of cocaine-conditioned locomotion (CHAPTER 3). On the other hand, nimodipine appeared to block the expression of cocaine-induced sensitization (CHAPTER 3).

The motor stimulant effects of cocaine can be conditioned to contextual stimuli (CHAPTER 2; Beninger and Herz, 1986; Pickens and Crowder, 1967; Schiff, 1982). The reinforcing effects of cocaine can also be conditioned. It has been demonstrated that after several administrations of a psychomotor stimulant drug paired with a specific environment, alternating with vehicle administrations paired with another environment, animals will spend more time in the environment that was previously associated with the drug, when given a choice between the two environments. This phenomenon is known as conditioned place preferences (CPP). CPP with psychomotor stimulants such as amphetamine, cocaine, methylphenidate, and nomifensine have been demonstrated (Di Scala et

3 A version of this chapter has been submitted to *Behavioural Pharmacology*.

al., 1985; Hiroi and White, 1990; Isaac et al., 1989; Martin-Iverson et al., 1985; Mithani et al., 1986; Spyraki et al., 1982). The dihydropyridine L-type Ca^{2+} channel antagonist, isradipine, has blocked cocaine-CPP at the highest of 3 non-sedating doses (Pani et al., 1991). It is necessary to replicate this finding with another dihydropyridine L-type Ca^{2+} channel antagonist to ensure that the blockade of cocaine-induced CPP is due to actions on the dihydropyridine-sensitive site on the L-type Ca^{2+} channel, and not to other effects of isradipine. Nimodipine was the drug of choice for this because of the previous work on this compound in the conditioning of psychomotor effects (DiLullo and Martin-Iverson, 1992b; Martin-Iverson et al., 1993; CHAPTER 2; CHAPTER 3). Therefore, we examined the ability of nimodipine to block the conditioned reinforcing effects of cocaine at 3 non-sedating doses.

4.2. MATERIALS AND METHODS

4.2.1. SUBJECTS

The subjects were 96 male Sprague-Dawley rats weighing between 250 and 350 grams, and were obtained from Health Sciences Animal Services, University of Alberta. They were on a 12 h light-dark cycle (lights on from 0700 to 1900) and were tested at the same time each day (from 1100 to 1600). Rats were housed in pairs in shoe-box cages with aspen chip, and had free access to food and water. The home and testing environment were maintained with a temperature of 22° C and the humidity at 50%.

4.2.2. DRUGS

The drugs were cocaine-hydrochloride (0 and 10 mg/kg/ml IP, British Drug Houses Inc.), dissolved in double-distilled water, and nimodipine (0, 0.1, 1.0, and 10 mg/kg/ml SC, courtesy of Miles Pharmaceuticals Inc.), dissolved in polyethylene glycol 400. Cocaine was injected immediately prior to placement of the animals in the test boxes, and nimodipine was injected 80 minutes prior to cocaine. Doses and drug regimen were determined from prior work in this laboratory (DiLullo and Martin-Iverson, 1992b; CHAPTER 2; CHAPTER 3).

4.2.3. APPARATUS

Eight CPP boxes (Acadia Instruments, Saskatoon, Saskatchewan, Canada) were used. These boxes had two compartments, with each compartment (30 L x 30 W x 25 H cm) consisting of a distinctive floor (unique tactile cues) and clear plexiglass sides. One floor was a stainless steel mesh with 1 cm squares and the other floor consisted of 14 horizontal stainless steel bars spaced 1.25 cm apart. The two compartments were separated by a partition containing an 7.5 cm long tunnel to allow animals access to both compartments, and the tunnels had a removable door on either end. Each compartment was transected by two infrared photobeams 3 cm above the floor that measured locomotor behaviour on each side, and by eight infrared photobeams spaced 3 cm apart that transected the compartments 15 cm above the floor to assess rearing behaviour in each compartment. The compartments rested on a fulcrum such that the compartment tilted 2 mm if an animal crossed from one compartment to the other. A weight of 50 gm near the entrance was sufficient to tilt the box. Tilting of a compartment broke an additional

photobeam such that the time spent in each compartment could be accurately determined. The photobeam arrays were connected to a computer, and interruptions of photobeams were counted by Turbo C software.

4.2.4. PROCEDURE

The procedures for drug-induced CPP closely followed those already established (Martin-Iverson et al., 1985; Mithani et al., 1986). Prior to being placed in the CPP boxes, rats were habituated to their home cages for 7 days. The animals were then randomly divided into eight groups of 12 each. There were four vehicle groups and four cocaine groups, with each of the four cocaine or vehicle groups receiving a separate dose of nimodipine, including vehicle. The allocation of animals to experimental conditions within each group was completely counterbalanced so that an equal number of animals received injections on each floor type and on each side of the room (i.e., half of each floor type was closer to the wall of the testing room, and half of each floor type was closer to the middle of the room). The test environment was illuminated with infrared light, extending into the visible red frequency. Throughout the experiment, the cages were cleaned between runs with an ammonia-based cleaner (Safeway Brand) that was mixed 6 parts water to 1 part cleaner.

The first 3 days of the experiment (part 1) were to habituate the animals to the CPP boxes and to determine initial side preferences, if any, by allowing them free access to both compartments for 30 minutes per day. In part 2, rats were conditioned to one of the compartments of the cages. Rats were given a cocaine or vehicle injection prior to confinement in one compartment of the cage for 30 minutes every second day. On alternate

days, all rats were injected with vehicle prior to confinement in the second compartment for 30 minutes. This totalled 8 drug injections (days 4,6,8,10,12,14,16, and 18) and 8 vehicle injections (days 5,7,9,11,13,15,17, and 19). The animals were then given a two day rest in their home cages to allow for drug clearance. Part 3 was the test day for CPP, where rats were injected with vehicle and then allowed free access to both compartments of the cage for 30 minutes. CPP were said to occur if rats spent more time in the compartment previously associated with the drug, relative to the pretest time. Part 4 was a test for place preferences while animals were under the influence of cocaine, and was done on the day following part 3. In this test, the central dividers were removed and all animals were injected with a challenge dose of 10 mg/kg cocaine followed by a 30 minute test.

4.2.5. STATISTICS

Data were analysed by analysis of variance (ANOVA). ANOVAs had three independent factors, side (2 levels: drug or vehicle), cocaine dose (2 levels: 0 or 10 mg/kg), and dose of nimodipine (4 levels: 0, 0.1, 1.0, and 10 mg/kg). There was also a repeated factor [days with 8 or 2 levels, depending on whether data from conditioning (8), CPP test (2), or the cocaine challenge test (2) were being analysed]. Data with more than 2 repeated factors were subjected to a variety of multivariate tests of significance to correct for unreliability of ANOVA due to lack of homogeneity of covariances, as is standard procedure with the statistical software used [Statistical Package for the Social Sciences for the PC (SPSSPC)]. ANOVA results are reported as significant only when verified by these additional tests. Significant main effects and interactions were followed by planned

individual comparisons by the F-test for multiple comparisons, the critical level of significance being $p < 0.05$ (Kiess, 1989).

4.3. RESULTS

4.3.1. CONDITIONED PLACE PREFERENCES

Each group served as its own control, allowing a comparison of the time spent in the "drug" compartment on the third pretest day to the time spent in the same compartment on the test day after conditioning. Table I contains the mean times spent in the drug-associated compartment on habituation day 3. ANOVA of the data from day 3 of habituation and from the conditioning test (day 20), revealed cocaine x days ($F(1,88)=9.45$, $p=0.003$) and nimodipine x days ($F(3,88)=3.18$, $p=0.028$) interactions. Individual comparisons showed that only animals that had previously received the combination of cocaine and vehicle (in place of nimodipine) significantly increased the time spent in the drug compartment (Figure 13). All three doses of nimodipine (0.1, 0, and 10 mg/kg) blocked cocaine-CPP, when rats were injected with vehicle and allowed to move freely between the two compartments of the cages in a 30 minute test.

4.3.2. COCAINE CHALLENGE TEST

When all animals received a challenge injection of cocaine (10 mg/kg), followed by free access to both compartments, ANOVA showed a cocaine x days interaction ($F(1,88)=29.94$, $p<0.001$). Individual comparisons revealed that groups previously receiving the combination of cocaine alone or cocaine with 0.1 or 10 mg/kg of nimodipine spent more time in the cocaine-paired compartment relative to the third habituation day.

Table I.

Mean time (sec) spent in the drug compartment on pretest day 3 (±SEM).

NIMODIPINE	0 mg/kg	0.1 mg/kg	1.0 mg/kg	10 mg/kg
VEHICLE	722 ± 170	928 ± 163	888 ± 174	798 ± 168
COCAINE	638 ± 169	886 ± 153	1008 ± 157	709 ± 163

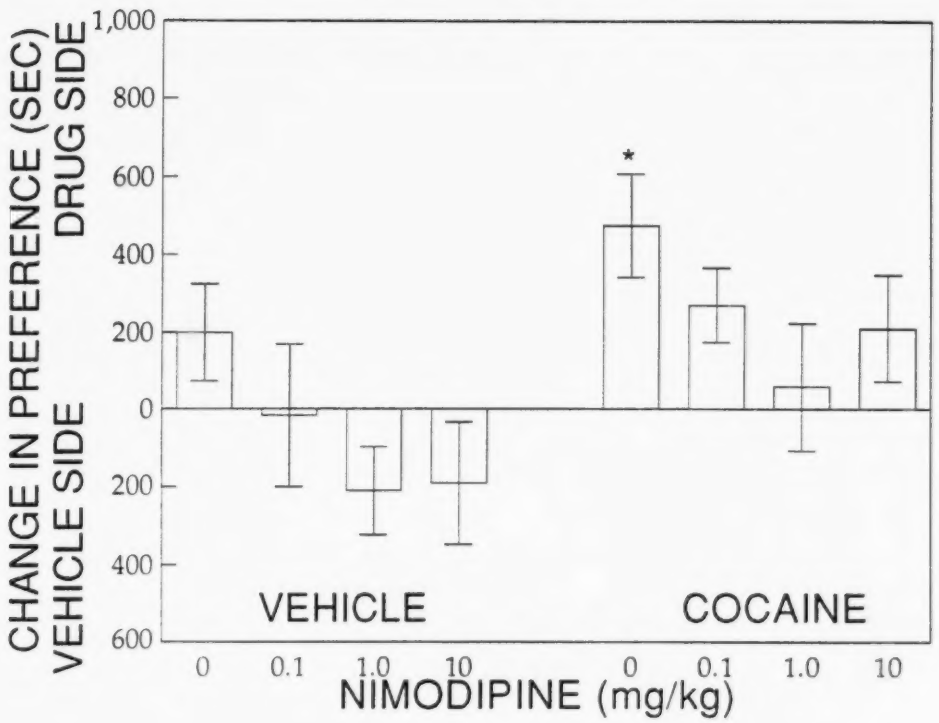

Figure 13. Conditioned place preferences to the cocaine-associated compartment comparing the change in time (sec) spent in the cocaine- (drug side) and vehicle-associated (vehicle side) compartments on a non-drug test day, after 8 pairings of a cocaine injection with confinement in the cocaine compartment, to the amount of time observed on a predrug day (habituation day 3). Cocaine (10 mg/kg) produced CPP, and this was blocked by all doses of nimodipine. Error bars represent ± SEM of each group. * Significantly different from pretest, $p < 0.05$ (n=12).

Cocaine-CPP was only blocked in the animals that had previously received 1.0 mg/kg nimodipine (Figure 14).

4.3.3. HORIZONTAL AND VERTICAL ACTIVITY, DAYS 4-19

During the conditioning period, only cocaine significantly affected horizontal activity ($F(1,88)=124.53$; $p<0.001$). Individual comparisons showed that there were no differences on horizontal activity as an average over the 8 treatment days among doses of nimodipine within vehicle- or within cocaine-conditioned groups (Figure 15).

Similarly, vertical activity during the conditioning period was only affected by cocaine ($F(1,88)=4.11$, $p=0.046$). Individual comparisons showed no differences between nimodipine dose within vehicle-conditioned groups, but vertical activity in the cocaine-treated group receiving 10 mg/kg nimodipine was significantly less than cocaine-conditioned groups receiving vehicle or 0.1 mg/kg nimodipine (Figure 16). Individual comparisons showed that only the group receiving 0.1 mg/kg dose of nimodipine together with cocaine had more vertical activity than their respective vehicle group.

These results indicate that nimodipine did not have a sedative effect on vehicle groups, nor did it significantly reduce cocaine-induced horizontal activity, but nimodipine did decrease the effect of cocaine on vertical activity.

4.4. DISCUSSION

The conditioning of the reinforcing actions of cocaine to contextual stimuli was prevented by nonsedating doses of nimodipine, a dihydropyridine L-type Ca^{2+} channel antagonist. All three doses of nimodipine (0.1, 1.0, and 10 mg/kg) were effective. On the

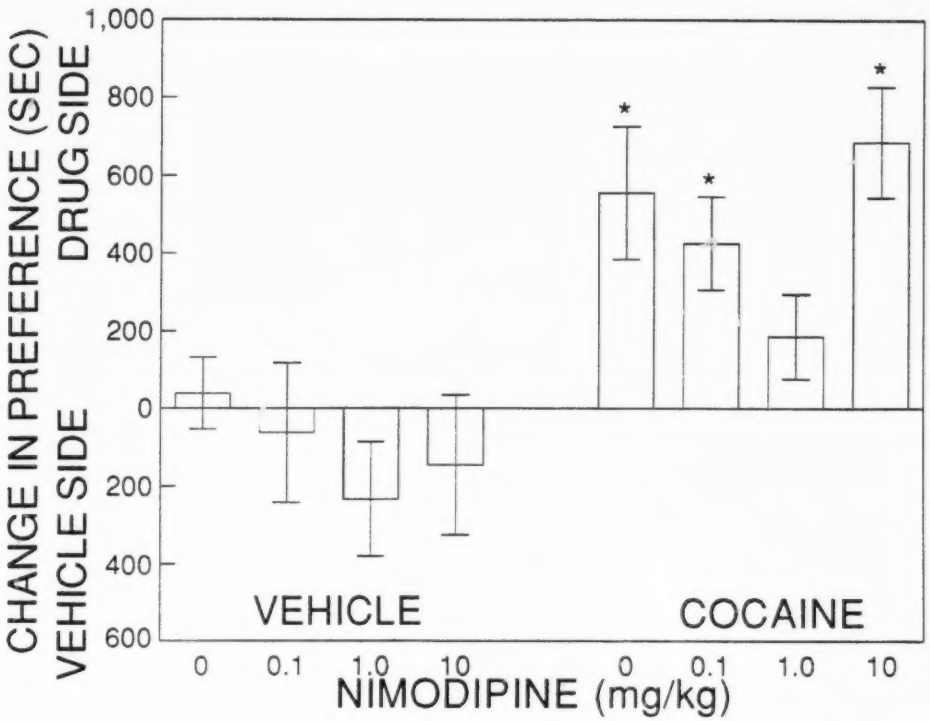

Figure 14. Conditioned place preferences to the cocaine-associated compartment, comparing the change in time (sec) spent in the cocaine- (drug side) and vehicle-associated (vehicle side) compartments, following a challenge injection of cocaine (10 mg/kg) (on the day following the CPP test), to the amount of time observed on a predrug day (habituation day 3). Cocaine produced CPP, and this effect was blocked by the second highest dose of nimodipine. Error bars represent ± SEM of each group. * Significantly different from pretest, $p < 0.05$ (n=12).

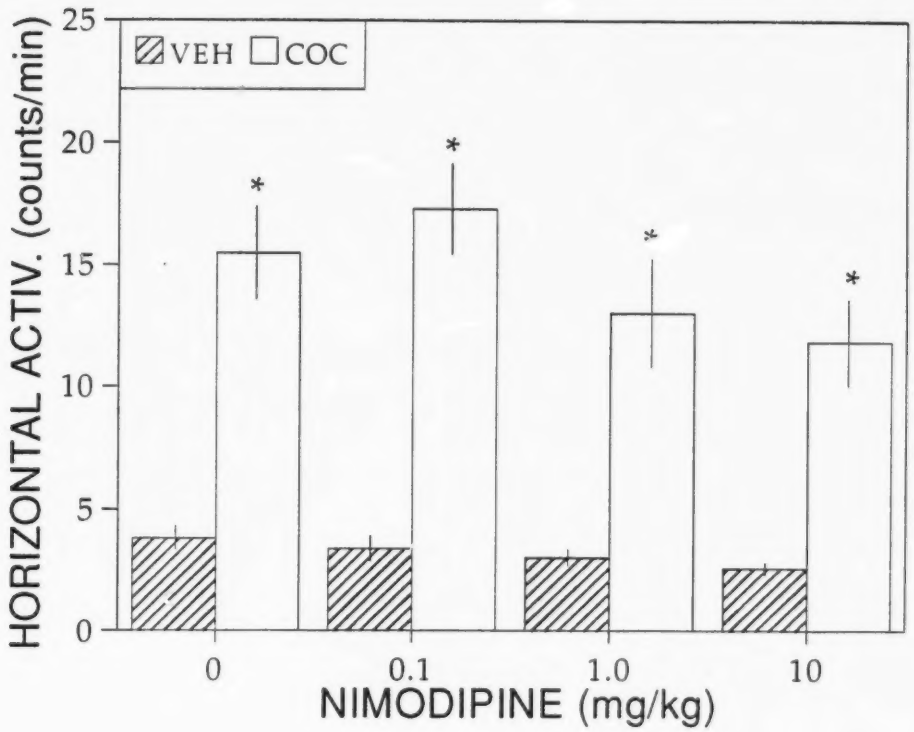

Figure 15. Average daily locomotor activity (counts/min) in the "drug"-associated compartment over the 8 conditioning days. Groups receiving cocaine (COC) were significantly more active than those receiving vehicle (VEH), but nimodipine had no significant effect at any dose. * Significantly different from appropriate vehicle and nimodipine control, $p < 0.05$ (n=12).

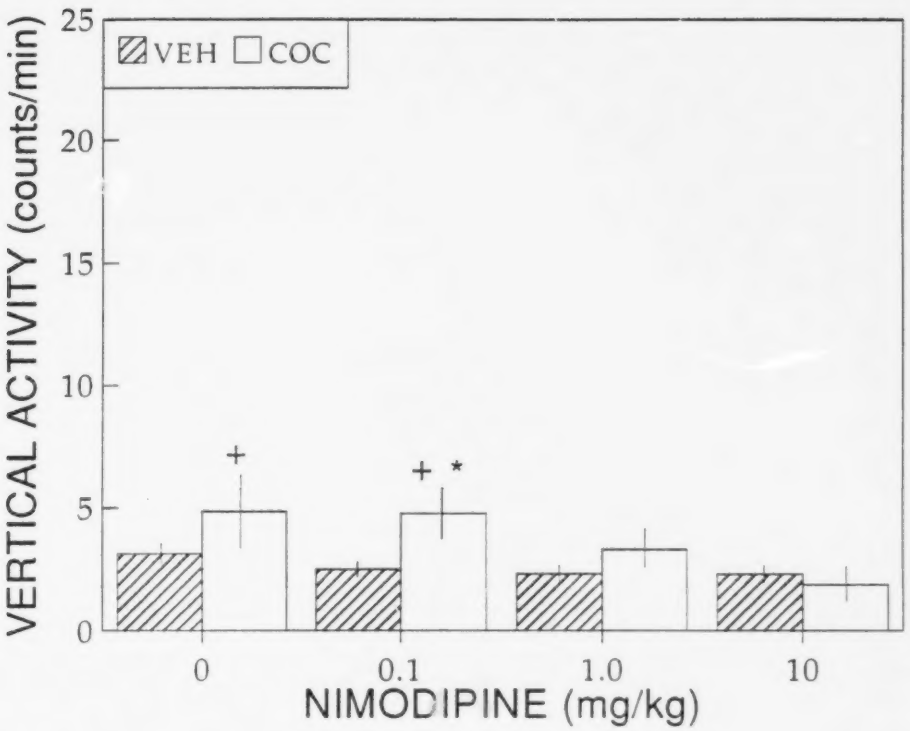

Figure 16. Average daily rearing activity (counts/min) in the "drug"-associated compartment over the 8 conditioning days. Groups receiving cocaine (COC) were significantly more active than those receiving vehicle (VEH). Nimodipine reduced the effect of cocaine on rearing in the 10 mg/kg group compared to the 0 and 0.1 mg/kg groups of nimodipine. * Significantly different from appropriate vehicle and nimodipine control, $p < 0.05$. + Significantly different from cocaine group receiving 10 mg/kg nimodipine, $p < 0.05$ ($n=12$).

following day, after a challenge injection of cocaine (10 mg/kg), place preferences were still blocked in the group of rats that had received 1.0 mg/kg nimodipine during conditioning.

There was a non-significant trend toward conditioned place aversion in vehicle-treated animals that received 1.0 or 10 mg/kg nimodipine during conditioning. The decreases induced by nimodipine in cocaine-conditioned rats were far greater than the decreases induced by nimodipine in vehicle-conditioned rats. This indicates that there was not a simple additive action of the nonsignificant aversive effects of nimodipine and reinforcing effects of cocaine. Thus, nimodipine appeared to block the reinforcing actions of cocaine. This conclusion is supported by a study with isradipine, another dihydropyridine L-type Ca^{2+} channel antagonist, to which neither conditioned place preferences nor place aversions occurred (Calcagnetti and Schecter, 1994).

The most effective dose of nimodipine appeared to be 1.0 mg/kg, since CPP was still blocked at this dose in rats that had received a challenge injection of cocaine. The three doses used formed an inverted U dose-response in the blockade of cocaine-CPP after a cocaine challenge. Similar inverted U-shaped dose-response functions have been observed with nimodipine in a variety of behavioural tasks, including blockade of cocaine drug discrimination (Cunningham et al., 1990), facilitation of eyelid condtioning in rabbits (Thompson et al., 1990; Disterhoft, 1990), and facilitation of 8-arm radial maze performance in rats (LeVere et al., 1990). At this time, a satisfactory explanation of the mechanisms for these dose-response relationships has not been formulated.

Nimodipine did not result in significant differences in horizontal or vertical activity between vehicle groups, although the 10 mg/kg dose of nimodipine did decrease vertical activity in cocaine groups. Therefore, none of the doses of nimodipine resulted in a sedative effect, although the high dose of nimodipine appeared to block the effect of cocaine on vertical activity. Unlike our results, the dose of isradipine that was effective in blocking cocaine (10 mg/kg) CPP (Pani et al., 1991) also blocked cocaine-induced motor activity (Pani et al., 1990a,b), without significantly reducing motor activity in vehicle rats (Pani et al., 1991). However, the 10 mg/kg dose of nimodipine did not block cocaine-induced horizontal activity in the present experiment, but was effective in blocking cocaine-induced motor activity at the 10 mg/kg dose in another study (Pani et al., 1990b). Differences in the types of test boxes used to measure motor activity in the experiments may account for the differences from our results. Pani et al. (1991) did not administer a challenge injection of cocaine following the CPP test, so it is difficult to know if the 2.5 mg/kg dose of isradipine would have blocked cocaine-CPP in a cocaine challenge test.

The precise nature of the role of L-type Ca^{2+} channels in the conditioning of psychomotor stimulants effects is not clear. The effect of nimodipine on CPP may have been mediated by reduction of dopamine release. Past results have shown that isradipine and nimodipine reduce dopamine overflow in the ventral striatum and block the increase in motor activity induced by cocaine in freely moving rats (Pani et al., 1990a,b). In addition, the amount of dopamine available for binding with dopamine receptors after cocaine administration is decreased by nimodipine (Burger and Martin-Iverson, 1994; Martin-Iverson and Burger, 1995). On the other hand, dopamine appears to activate L-type

Ca^{2+} channels. In bass retinal horizontal cells, dopamine or a cAMP analogue potentiated the L-type Ca^{2+} channel current. This effect was blocked by the D_1 receptor antagonist, SCH23390, and by protein kinase inhibitor, indicating that it occurs through activation of cAMP (Pfeiffer-Lin and Lasater, 1993). Amphetamine- (Hoffman and Beninger, 1989) and pipradrol-CPP (White and Hiroi, 1992) were blocked by the D_1 antagonist SCH23390, possibly through D_1-receptor mediated effects on L-type Ca^{2+} channels. Interestingly, the conditioned horizontal motor effects of amphetamine can be blocked with nimodipine, but only when given with a D_2 receptor antagonist (DiLullo and Martin-Iverson, 1992b). These results may indicate an effect of nimodipine on cells postsynaptic to dopamine neurons.

The present results confirm those from another study with isradipine, in which cocaine-CPP were blocked (Pani et al., 1991), and supports the hypothesis that L-type Ca^{2+} channel antagonists may block the reinforcing effects of cocaine. These results also support past results showing that the establishment and the expression of cocaine-conditioned horizontal activity can be blocked by nimodipine alone, or by a combination of nimodipine and D_2 dopamine receptor antagonist, haloperidol, respectively (CHAPTER 2; CHAPTER 3). Whether the effects of nimodipine on CPP is due to blockade of the direct reinforcing effects of cocaine or to the blockade of the conditioning of the reinforcing effects is not presently known. However, the ineffectiveness of nimodipine at reducing cocaine self-administration in rats (Goldberg et al., 1990) supports the latter mechanism (but see Kuzmin et al. (1992) for a report of blockade of self-administration of cocaine in mice). A recent clinical study did not find a reduction in cue-induced cocaine

craving in cocaine addicts treated with a single dose of nimodipine (Rosse et al., 1994). However, our data from rat studies suggest that co-administration of a dihydropyridine L-type Ca^{2+} channel antagonist and a D_2 dopamine receptor antagonist is necessary to block the *expression* of cocaine-conditioned motor stimulant effects (CHAPTER 3).

CHAPTER 5. CLASSICALLY CONDITIONED MOTOR EFFECTS DO NOT OCCUR WITH COCAINE IN AN UNBIASED CONDITIONED PLACE PREFERENCES PROCEDURE[4]

5.1. INTRODUCTION

The validity of the classical conditioning of psychomotor stimulant effects has recently been questioned. Much of the previous work in the determination of conditioned motor effects with psychomotor stimulants has been accomplished in single compartment procedures. When psychomotor stimulant administration is paired with a single compartment (the CS+) several times, and then the CS+ is presented to the animal on a drug free test day, an increase in locomotion and rearing, as measured by automated behaviour measurement devices, is elicited (Beninger and Hahn, 1983; Martin-Iverson and McManus, 1990; CHAPTER 2; CHAPTER 3; CHAPTER 4). Several studies have suggested that classical conditioning with psychomotor stimulants either does not occur (Gold et al., 1988; Damianopoulos and Carey, 1992; Szechtman et al., 1993), or that the classical conditioning of at least some motor behaviours, such as locomotion and rearing, measured by direct observation, does not occur (Martin-Iverson and Fawcett, in press). We have failed to observe cocaine-conditioned locomotion and rearing in a two compartment procedure using automated measurement similar to that which shows such effects in a single compartment (Reimer et al., unpublished results). During the conditioning procedure, rats received cocaine injections and were confined to one compart-

4 A version of this chapter has been submitted to *Behavioural Pharmacology*.

ment (the CS+) on even numbered days, and then received vehicle injections and were confined to another compartment(the CS-) on odd numbered days. On a drug free test day, when animals had access to both the CS+ and the CS-, locomotion and rearing activity were greatest in the CS- compartment. At the same time, cocaine-CPP were observed for the CS+ compartment.

An increase in the behavioural effects of the drug, known as behavioural sensitization, is observed when psychomotor stimulants are repeatedly administered. Although context-specificity plays an important role in sensitization (Post et al., 1981; Schiff, 1982; Barr et al., 1983), the importance of classical conditioning is unclear (Robinson and Becker, 1986; Martin-Iverson et al., 1988a; Martin-Iverson et al., 1988b; Baldo and Kelly, 1991; Martin-Iverson, 1991; Stewart and Vezina, 1991, Szechtman et al., 1993). Sensitization to amphetamine may have an associative and a non-associative component. For example, context-independent sensitization can occur in rats receiving continuous infusions of amphetamine or a direct dopamine D_2 receptor agonist through osmotic minipumps (Martin-Iverson et al., 1988a; Martin-Iverson et al., 1988b; Martin-Iverson, 1991). Intermittent injections of amphetamine resulted in context-dependent sensitization of locomotion and rearing; however, following extinction of conditioning, context-independent sensitization emerged for locomotion but not for rearing (Stewart and Vezina, 1991; but also see Ahmed et al., 1993). Intermittent injections of D_2/D_3 receptor agonist, quinpirole, indicated that the sensitization was behaviour-specific, and not environment-specific (Willner et al., 1992), while another study with quinpirole suggested that the context-specific component of sensitization was about 50%, the behavioural

component 30%, and the context-independent component 20% (Szechtman et al., 1993). Cocaine-induced sensitization has been shown to be largely context-dependent (Post et al., 1981; Weiss et al., 1989; CHAPTER 2). However, sensitization and putative classical conditioning of cocaine's effects may be pharmacologically dissociated, both in development (CHAPTER 2) and in expression (CHAPTER 3).

There are therefore two major issues concerning context-specificity of the effects of repeated administration with psychomotor stimulants that have yet to be resolved. One is whether or not classical conditioning provides an adequate explanation or mechanism for the changes in behaviours observed in the environment previously associated with stimulant treatments. The second is the degree to which classical conditioning determines sensitization to psychomotor stimulants.

The present study was designed to further examine classical conditioning and sensitization in a two-compartment box. Two experiments were involved. Both experiments used four groups of rats. Two groups were conditioned with cocaine in the CS+ compartment and vehicle in the CS- compartment, and two groups were conditioned with cocaine or vehicle and access to both compartments (the tunnel separating the 2 compartments was left open). Each experimental group was paired with a vehicle-treated control group. In experiment 1, animals were habituated to the test boxes prior to the conditioning period, as is often done when testing for CPP, and in experiment 2, there was no habituation to the test boxes prior to the conditioning period. First, we wanted to determine if a previous finding of increased activity in the CS- compartment on a non-drug test day could be replicated. Second, some groups were trained with the tunnel always

open to test the possibility that the apparent lack of conditioning was due to testing the animals with the tunnel open when they had been trained with the tunnel closed. Third, it was possible that latent inhibition played a role in the absence of conditioned motor effects in the CS+ compartment, i.e., prior habituation to the test boxes in experiment 1 could have resulted in latent inhibition, since rats were initially exposed to the CS+ compartment in the absence of the unconditioned stimulus. Latent inhibition should result in less effective conditioning, if the behaviour being considered can be classically conditioned. To determine if latent inhibition was important, habituation (experiment 1) versus no habituation (experiment 2) was considered. Finally, we examined the development of sensitization during training, and the expression of sensitization after extinction with the tunnel open or closed. Thus, the role of context-specificity, the effects of extinction, and the effects of habituation versus no habituation in classical conditioning and behavioural sensitization were examined in a one-compartment (open tunnel) versus a two-compartment procedure (closed tunnel).

5.2. METHODS

5.2.1. SUBJECTS

The subjects were 96 male Sprague-Dawley rats weighing between 250 and 350 grams, and were obtained from Health Sciences Animal Services, University of Alberta. They were on a 12 h light-dark cycle (0700 to 1900) and were tested at the same time each day (1100 to 1700 h). Rats were housed in pairs in shoe-box cages with aspen chips, and had free access to food and water, except during testing. The home and testing

environment were maintained with a temperature of 21.9-22.3°C and the humidity at 39.5-40.5%. Two rats in a cocaine treatment group died, and their data were not included in the analysis. Post mortem examination suggested that one rat died of cardiovascular collapse, and the second had left ventricular hypertrophy, which may have contributed to its death.

5.2.2. DRUGS

Cocaine-hydrochloride (7.5 or 10 mg/kg/ml IP, British Drug Houses Inc.) was dissolved in double-distilled water. Cocaine or vehicle was injected immediately prior to placement of the animals in the test boxes. Dose and drug regimen was determined from prior work (CHAPTER 2,3).

5.2.3. APPARATUS

Eight conditioned place preferences (CPP) boxes (Acadia Instruments, Saskatoon, Saskatchewan, Canada) were used. These boxes had two compartments, with each compartment (30 L x 30 W x 25 H cm) consisting of a distinctive floor (unique tactile cues) and clear plexiglass sides. One floor was a stainless steel mesh with 1 cm squares and the other floor consisted of 14 horizontal stainless steel bars spaced 1.25 cm apart. The two compartments were separated by a partition containing a 7.5 cm long tunnel to allow animals access to both compartments, and the tunnels had a removable door on either end. The boxes were built with a tunnel for practical reasons, i.e., because a tilt box timing mechanism was responsible for counting the time spent in each compartment. It was thought that the presence of a tunnel may be more conducive to the rat "choosing" one compartment or the other, rather than remaining between the two compartments and

rocking the box back and forth. Each compartment was transected by two infrared photobeams 3 cm above the floor that measured locomotor behaviour on each side, and by eight infrared photobeams spaced 3 cm apart that transected the compartments 15 cm above the floor to assess rearing behaviour in each compartment. The compartments rested on a fulcrum such that the compartment tilted 2 mm if an animal crossed from one compartment to the other. A weight of 50 gm near the entrance was sufficient to tilt the box. Tilting of a compartment broke an additional photobeam such that the time spent in each compartment could be accurately determined. The photobeam arrays were connected to a computer, and interruptions of photobeams were counted by Turbo C software.

5.2.4. PROCEDURE

5.2.4.1. Experiment 1 (prior habituation to the test boxes):

The procedures for drug-induced CPP essentially followed those already established (Martin-Iverson et al., 1985; Mithani et al., 1986). Prior to being placed in the CPP boxes, rats were habituated to their home cages for 7 days. The animals were then randomly divided into 4 groups of 12 each. There were 2 vehicle groups and 2 cocaine groups. Groups 1 and 2 included one vehicle and one cocaine group that were conditioned with access restricted to one compartment of the test boxes. Groups 3 and 4 included one vehicle and one cocaine group that were conditioned with the tunnel that separated the two compartments left open. Each group was completely counterbalanced such that, following injections, an equal number of rats from each group were placed on each floor type, on each side of the room, i.e., in each test box, half of each floor type was closer to the wall of the testing room, and half of each floor type was closer to the middle of the room. The

test environment was illuminated with infrared light, extending into the visible red frequency. Throughout the experiment, the cages were cleaned between runs with an ammonia-based cleaner (Safeway Brand) that was mixed 6 parts water to 1 part cleaner.

The first 4 days of the experiment (part 1) were to habituate the rats to the CPP boxes by allowing them free access to both compartments for 30 minutes per day. Part 2 was the conditioning procedure, which also lasted 30 minutes per day. In groups 1 and 2, rats received cocaine or vehicle on odd numbered days and were restricted to the CS+ compartment. On even numbered days animals received vehicle injections and were confined to the CS-. The treatment totals were 8 drug injections (days 5,7,9,11,13,15,17, and 19) and 8 vehicle injections (days 6,8,10,12,14,16,18 and 20). Part 3 (day 21) was the test day for CPP, where rats were injected with vehicle and then allowed free access to both compartments of the cage for 30 minutes. CPP were said to occur if cocaine-treated groups spent significantly more time in the CS+ compartment than did the vehicle-treated controls. Part 4 was the extinction period, when animals received the same treatment as during the conditioning period, except that vehicle was substituted for cocaine in the CS+ compartment. Extinction lasted 22 days, 11 exposures each to the CS+ and the CS-. Part 5 was the test to determine if extinction had occurred. The procedure was identical to part 3. Part 6 was the test for behavioural sensitization, and was done on the 3 days following part 5. All animals were injected with 7.5 mg/kg cocaine and tested for 30 minutes. On the first two days, animals were restricted to either the CS+ or the CS-, an equal number in each condition on each day. On the third day, the central dividers were removed for the test.

Groups 3 and 4 were conditioned and tested with the tunnel open, but were subjected to the same number of days of treatment as groups 1 and 2. On vehicle days, groups 3 and 4 received injections outside of the room where the test boxes were. All other conditions for these groups were identical to those of groups 1 and 2 respectively.

5.2.4.2. Experiment 2 (no prior habituation to the test boxes):

Experiment 2 also involved 4 groups of rats and utilized the same basic procedures and test days as experiment 1. The differences between experiment 2 and experiment 1 included no prior habituation to the test boxes, and only 16 days of extinction.

5.2.5. STATISTICS

All data were analysed by analysis of variance (ANOVA). In the CPP tests, there were 3 independent factors, compartment (2 levels: CS+ or CS-), habituation (2 levels: present or absent), and cocaine dose (0 or 10 mg/kg). In the drug compartment (CS+) during conditioning and extinction, the horizontal and vertical activity data had 2 independent factors, tunnel (2 levels: open or closed) and cocaine dose (2 levels: 0 or 10 mg/kg). In the vehicle compartment (CS-) during extinction, the horizontal and vertical activity data had 1 independent factor, cocaine dose (2 levels: 0 or 10 mg/kg). There was also a repeated factor (days with 8 or 11 levels (8 levels in experiment 2), depending on whether data were from the conditioning or extinction periods respectively). On the test days for conditioned motor activity, extinction, and for behavioural sensitization in groups conditioned with the tunnels closed, there were 3 independent variables, habituation (2 levels: present or absent), compartment (2 levels: CS+ and CS-), and cocaine dose (2 levels: 0 or 10 mg/kg). The data from these tests only included the first 5 minutes of the

30 minute test, except for the challenge test with the tunnels closed, which included the entire 30 minutes. On the test day for conditioned motor effects, extinction, and behavioural sensitization in the groups conditioned with the tunnels closed, there were 2 independent variables, habituation (2 levels: present or absent) and cocaine dose (2 levels: 0 or 10 mg/kg). Data with more than 2 repeated factors were subjected to a variety of multivariate tests of significance to correct for unreliability due to lack of homogeneity of covariances, as is standard procedure with the statistical software used (Statistical Package for the Social Sciences for the PC (SPSSPC)). Significant ANOVA results are reported in this paper only when verified by these additional tests. Significant main effects and interactions were followed by individual comparisons by the F-test for multiple comparisons, the critical level of significance being $p < 0.05$.

5.3. RESULTS

5.3.1. CONDITIONED PLACE PREFERENCES

The total time in the test boxes during the CPP test was 1800 sec. Rats conditioned with cocaine in the CS+ compartment displayed a significantly greater amount of time in the CS+ compartment on a drug free test day than did vehicle-conditioned rats. There was a main effect of drug ($F(1,44)=24.85$, $p=0.001$). This effect was extinguished in the cocaine group after several presentations (11 (experiment 1) or 8 (experiment 2)) of the CS+ in the absence of cocaine (Figure 17). There were no significant effects of habituation, so both habituated ($n=12$) and non-habituated groups ($n=12$) were analysed together ($n=24$).

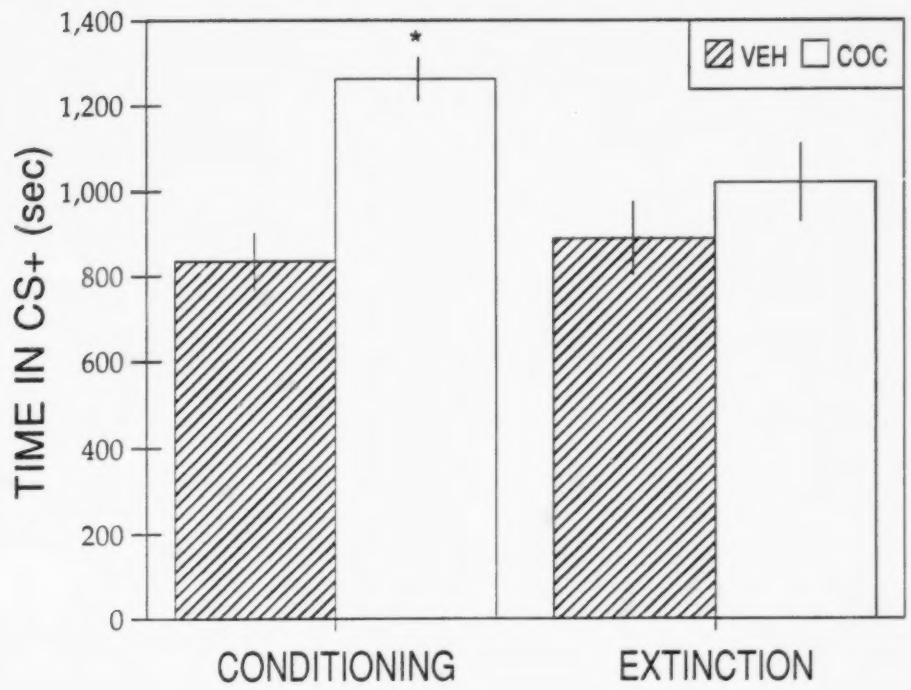

Figure 17. Conditioned place preferences to the cocaine-associated compartment in all rats conditioned with the tunnel closed, comparing the time spent by cocaine- (COC) or vehicle- (VEH) trained groups in the cocaine-associated compartment (CS+) on a non-drug test day. COC (10 mg/kg) produced CPP, and this effect was extinguished following several pairings of vehicle injections with confinement in the cocaine-associated compartment (the CS+) and the vehicle-associated compartment. Total test duration was 1800 sec. Error bars represent ± SEM of each group. * Significantly different from respective vehicle group, $p < 0.05$, multiple F-test (n=24).

5.3.2. HORIZONTAL AND VERTICAL ACTIVITY DURING CONDITIONING

(All horizontal and vertical activity is expressed as a rate (counts/min) as detected by photobeam interruptions). Figure 18A shows the results from the conditioning days for horizontal activity, comparing activity in habituated groups conditioned with the tunnel closed to those conditioned with the tunnel open. Rats in the tunnel open condition had significantly more horizontal activity, although both cocaine groups displayed behavioural sensitization over the 16 days, indicated by a tunnel x drug x days interaction ($F(7,294)=2.49$, $p=0.017$). For vertical activity (Figure 18C), individual comparisons revealed that vehicle animals in the open tunnel group were more active than other groups, as were the vehicle compared to cocaine groups. There were main effects of tunnel ($F(1,42)=9.06$, $p=0.004$) and of drug ($F(1,42)=4.7$, $p=0.036$).

The results for horizontal activity from the conditioning days in non-habituated groups are displayed in Figure 18B. Open groups were the most active, but cocaine groups were more active than respective vehicle groups, and this effect increased over days. There was a tunnel x drug x days interaction ($F(7,308)=3.86$, $p<0.001$). For vertical activity (Figure 18D), vehicle rats from the open group were the most active, and this effect increased over days. There was a tunnel x days interaction ($F(7,308)=3.69$, $p=0.001$).

5.3.3. HORIZONTAL ACTIVITY IN THE CS+ VERSUS THE CS- IN GROUPS CONDITIONED WITH TUNNEL CLOSED

On the test day for conditioned motor activity, horizontal and vertical activity was greatest in the CS- compartment. However, there was no effect of habituation so the data was pooled (horizontal activity (counts/min): CS+ = 17.05 ± 1.36 [vehicle]; 15.71 ± 1.11

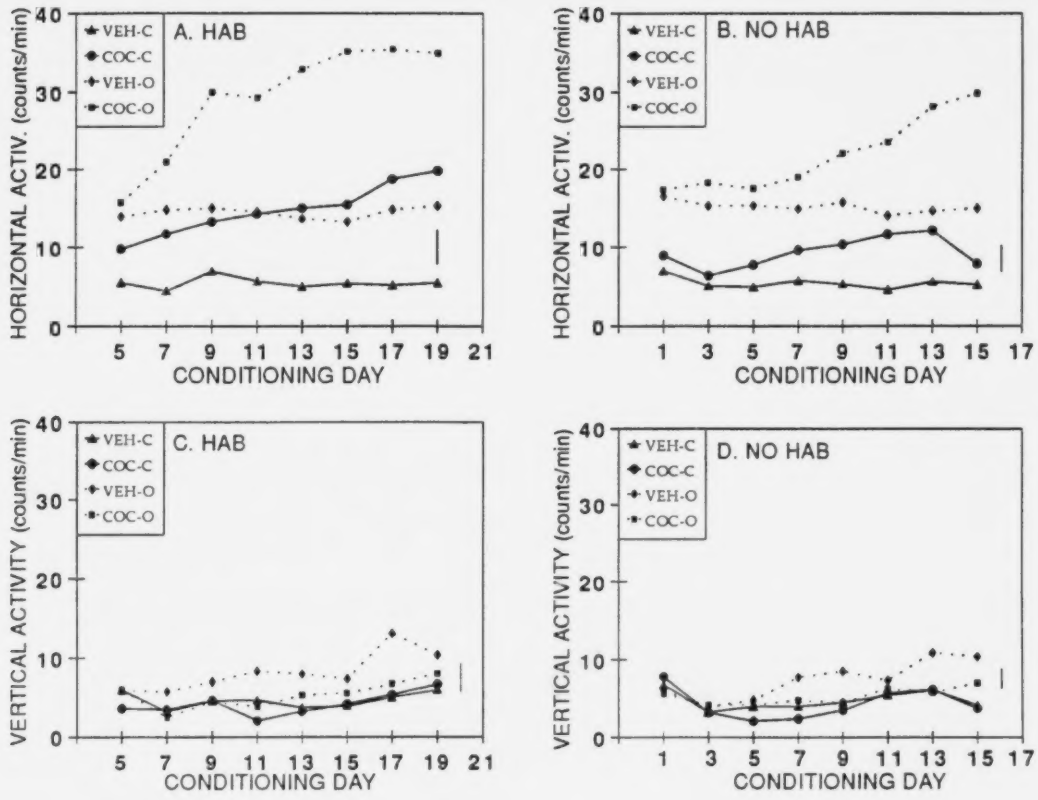

Figure 18.

(A) Mean horizontal activity (counts/min) from 30 min daily training trials in the cocaine-associated compartment (CS+) on the 8 drug conditioning days in rats habituated to the test boxes prior to conditioning (HAB), comparing the horizontal activity in groups conditioned with cocaine (COC) or vehicle (VEH) with the tunnel closed (C) to those conditioned with the tunnel open (O). For example, the VEH-O group was conditioned with vehicle and the tunnel open. Both groups in the open condition were more active than their respective group in the closed condition, and both cocaine-conditioned groups displayed sensitization. The critical difference at $p < 0.05$ is 4.38, multiple F-test (bar in lower right corner). Legends continued on following page.

(B) Mean horizontal activity (counts/min) in COC and VEH groups conditioned with the tunnel closed on the test day for extinction of conditioned activity. Activity rates are from the first 5 minutes spent in each of the CS+ and the CS- compartments, when animals had free access to both compartments. There were no significant differences between COC and VEH groups in the CS+ compartment, but the previously habituated COC group was more active than the VEH group in the CS- compartment. * Significantly different from VEH, $p < 0.05$, multiple F-test.

(C) Mean horizontal activity (counts/min) in groups conditioned with the tunnel closed on the cocaine challenge test day with the tunnel open. Activity rates are from the first 5 minutes spent in each of the CS+ and the CS- compartments, when animals had free access to both compartments. Following a cocaine (7.5 mg/kg) injection, the habituated COC group was more active than the VEH group in both the CS+ and the CS- compartments. There were no significant differences between COC and VEH groups that were not habituated prior to conditioning. * Significantly different from vehicle, $p < 0.05$, multiple F-test.

(D) Mean horizontal activity (counts/min) in groups conditioned with the tunnel closed on the cocaine challenge test days with the tunnel closed. Access was restricted to the CS+ or the CS- compartment of a two compartment box for two counterbalanced challenge tests with cocaine (7.5 mg/kg). Sensitization, as observed by an increase in locomotor activity (counts/min), occurred in the both the CS+ and the CS- compartment in the habituated COC group and in the CS- in the non-habituated COC group.

* Significantly different from respective vehicle group, $p < 0.05$ ($n=12$).

[cocaine]; CS- = 15.48±1.49 [vehicle]; 28.41±2.44 [cocaine]; vertical activity (counts/min): CS+ = 13.72±1.28 [vehicle]; 10.49±1.31 [cocaine]; CS- = 13.12±1.80 [vehicle]; 18.68±1.74 [cocaine]. It was considered possible that the increased rate of activity in the CS- compared to the CS+ compartment, during the entire 30 minute test, was due to differential duration of the sampling period. Horizontal activity of cocaine groups was assessed for a longer period of time in the CS+ than in the CS- compartment due to CPP. Since horizontal activity activity decreased as a function of time in these tests, it was possible that the differential activity in the CS+ and CS- compartments in the cocaine groups was due to this differential sampling duration. To avoid the effect of differential sampling duration, data from the first 5 minutes was analysed (Figure 19A), since this was the longest time that some rats spent in the CS- compartment. ANOVA showed that there was no effect of habituation. There were no significant differences in locomotor or rearing activity between the CS+ and the CS- in cocaine groups, in counts/min, for the first 5 minutes in each compartment, but cocaine groups were significantly more active than vehicle in both compartments in habituated and in non-habituated groups. There was a main effect of drug ($F(1,44)=10.41$, $p=0.002$). Figure 19B displays the results of the first 5 minutes of the test day following an extinction period. Rats were injected with vehicle and then exposed to both compartments during this test. There was a main effect of drug ($F(1,44)=5.45$, $p=0.024$), indicating that extinction did not occur. Individual comparisons showed that horizontal activity of habituated rats previously administered cocaine was higher in the CS- compartment than that of vehicle-treated rats. Figure 19C shows the results for the cocaine (7.5 mg/kg) challenge test with

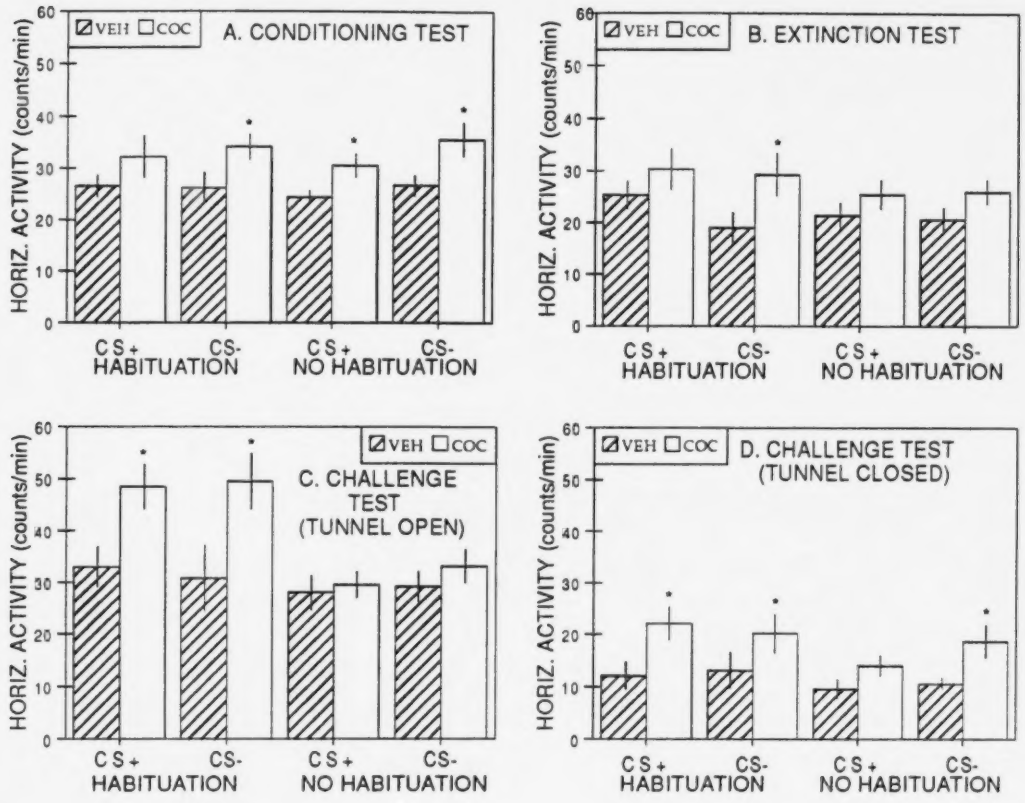

Figure 19.

(A) Mean horizontal activity (counts/min) in groups previously conditioned with cocaine (COC, 10 mg/kg, IP) or vehicle (VEH) with the tunnel closed on the test day for conditioned activity. Activity rates are from the first 5 minutes spent in each of the CS+ and the CS- compartments, when animals had free access to both compartments. There were no significant effects of habituation and no significant differences in activity between the CS+ and the CS- compartments in previously cocaine-conditioned rats, but cocaine-trained rats were significantly more active than vehicle-trained rats in both compartments (a significant main effect of drug, see text). * Significantly different from VEH, $p < 0.05$, Multiple F-test. Legends continued on following page.

(B) Mean horizontal activity (counts/min) in COC and VEH groups conditioned with the tunnel open on the test day for extinction of conditioned activity. Activity rates are from the entire 30 minute test. An increase in activity in was observed in the habituation COC group, but not in the no-habituation COC group. * Significantly different from vehicle, $p < 0.05$, multiple F-test.

(C) Mean horizontal activity (counts/min) in groups conditioned with the tunnel open on the cocaine challenge test day. Activity rates are from the entire 30 minute test. Following a cocaine (7.5 mg/kg) injection, there were no significant differences between any two groups.

(D) Mean horizontal activity (counts/min) in COC and VEH groups conditioned with the tunnel open, comparing the activity in habituated to that in non-habituated groups. Access was restricted to one compartment of a two compartment box for two challenge tests with cocaine (7.5 mg/kg), and then the average counts/min in the two compartments were calculated. Sensitization, as observed by an increase in locomotor activity (counts/min), occurred in the habituation COC group, but not in the no-habituation COC group. * Significantly different from respective vehicle group, $p < 0.05$ ($n=12$).

the tunnel open that followed the extinction period. Sensitization was only evident in rats that were habituated prior to the conditioning period, and this effect appeared in both compartments. ANOVA revealed main effects of habituation ($F(1,40)=7.48$, $p=0.009$) and prior drug treatment ($F(1,40)=6.69$, $p=0.013$). Figure 19D shows the results for the cocaine challenge tests when rats were restricted to either the CS+ or the CS- compartments. There was a main effect of prior drug treatment ($F(1,44)=8.75$, $p=0.005$), and habituated animals previously conditioned with cocaine had significantly more horizontal activity than vehicle-conditioned animals in both the CS+ and CS- compartments, but non-habituated cocaine-treated animals were only more active in the CS- compartment.

5.3.4. VERTICAL ACTIVITY IN THE CS+ VS THE CS-

There were no significant differences between any cocaine and vehicle groups in vertical activity in the first 5 minutes in the CS+ and the CS- compartments during the tests for conditioning, extinction, or following the cocaine challenge (data not shown).

5.3.5. HORIZONTAL ACTIVITY IN GROUPS CONDITIONED WITH TUNNEL OPEN

In Figure 20A shows the results for the conditioning test for groups conditioned with the tunnel open. Only the cocaine group that was habituated to the test boxes before training exhibited higher rates of horizontal activity than it's vehicle control group. There was an overall effect of drug ($F(1,44)=9.92$, $p=0.003$), and a trend towards an effect of habituation ($F(1,44)=3.97$, $p=0.052$). Figure 20B shows the results of the last day of the extinction test. Increased horizontal activity induced by cocaine did not extinguish in the

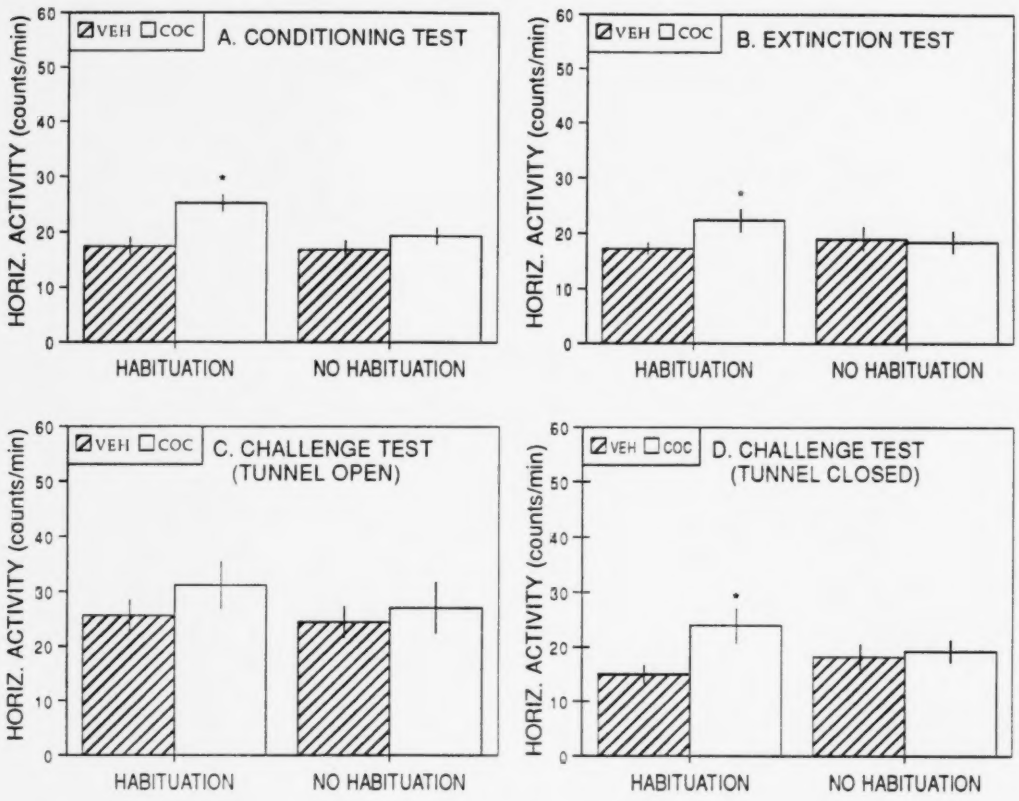

Figure 20.

(A) Mean horizontal activity (counts/min) in groups conditioned with the tunnel open on the test day for conditioned activity. Activity rates are from the entire 30 minute test. An increase in activity in the COC group was observed in habituated, but not in non-habituated rats. * Significantly different from vehicle, $p < 0.05$, Multiple F-test. Legends continued on following page.

(B) Mean vertical activity (counts/min) in the CS+ compartment during extinction days in rats habituated to test boxes prior to conditioning. Both COC-O and VEH-O groups were more active than COC-C and VEH-C groups, respectively. Differences in vertical activity between the COC-O and VEH-O groups declined over days, but differences between the COC-C and VEH-C groups arose over days. The C.D. at $p < 0.05$ is 3.42, multiple F-test (bar in lower right corner).

(C) Mean horizontal activity (counts/min) in the vehicle-associated compartment (CS-) during extinction days in rats habituated to test boxes prior to conditioning, comparing the horizontal activity in COC and VEH groups conditioned with the tunnel closed (C). Beginning on day 37, the COC-C group became significantly more active than the VEH-C group. The C.D. $p < 0.05$ is 1.81, multiple F-test (bar in lower right corner).

(D) Mean vertical activity (counts/min) in the CS- compartment during 11 extinction days in rats habituated to test boxes prior to conditioning, comparing the vertical activity in COC and VEH groups conditioned with the tunnel closed (C). The COC-C group had significantly more vertical activity than the VEH-C group. The C.D. at $p < 0.05$ is 3.08, multiple F-test (bar in lower right corner) [n=12].

habituated cocaine group. Since this day was a continuation of the tunnel open extinction procedure, see the "Extinction period (horizontal activity)" section below for the ANOVA of the extinction period in these groups. Figure 20C shows that there were no significant differences between groups in the cocaine challenge test with the tunnel open that followed the extinction period. On the cocaine challenge test when rats were restricted to one compartment, the habituated, but not the non-habituated group previously conditioned with cocaine displayed an increase in horizontal activity. There was a main effect of cocaine ($F(1,44)=4.44$, $p=0.041$, Figure 20D).

5.3.6. VERTICAL ACTIVITY IN GROUPS CONDITIONED WITH TUNNEL OPEN

On the test day for conditioned vertical activity, only habituated rats displayed an increase in vertical activity compared to respective vehicle groups. Only a main effect of habituation was significant according to ANOVA ($F(1,44)=4.35$, $p=0.043$). Individual comparisons revealed that this effect was only present in the habituated cocaine group with respect to non-habituated vehicle and cocaine. There were no significant differences in vertical activity between groups induced by the cocaine challenge (data not shown).

5.3.7. EXTINCTION PERIOD (HORIZONTAL ACTIVITY)

As previously described, horizontal and vertical activity rates were not higher in the CS+ than in the CS- compartment when the tunnel was open. However, when access was restricted to one compartment during the extinction test in rats previously habituated to the test boxes, individual comparisons showed that the rate of horizontal activity in the cocaine-treated group was greater than the vehicle group in the CS+ but not the CS-

compartment on most days (Figures 21A and 21C). In the CS+ compartment, there was a large main effect of tunnel ($F(1,42)=95.61$, $p<0.001$), indicating that rats were more active in the tunnel open condition, and of drug ($F(1,42)=11.75$, $p=0.001$), indicating that cocaine groups were more active than vehicle groups. There was also a small effect of days ($F(1,420)=1.88$, $p=0.046$), indicating a slight decrease in activity over time. On alternating days of extinction, the groups conditioned with the tunnel closed were injected with vehicle and restricted to the CS- compartment. There was a drug x days interaction ($F(10,200)=1.99$, $p=0.036$), indicating that rats previously conditioned with cocaine became more active while vehicle conditioned rats became less active over days, with no differences between groups except on days 37, 41, and 43 (Figure 21C). Thus, a cocaine effect in habituated rats only developed in the CS- compartment after 8 days of extinction.

For non-habituated groups, horizontal activity in previously cocaine-treated rats was higher than vehicle-treated rats on days 17, 22, and 27 in the CS+ compartment, but this difference extinguished (Figure 22A). The groups previously conditioned and then extinguished with the tunnel open were more active than the tunnel closed groups, as revealed by a large main effect of tunnel ($F(1,44)=85.06$, $p<0.001$). In the vehicle group in the tunnel open condition, activity increased over days, resulting in a main effect of days ($F(7,308)=3.07$, $p=0.004$). On alternating days (Figure 22C), when groups conditioned with the tunnel open received vehicle injections and were restricted to the CS- compartment, there were no significant differences between groups.

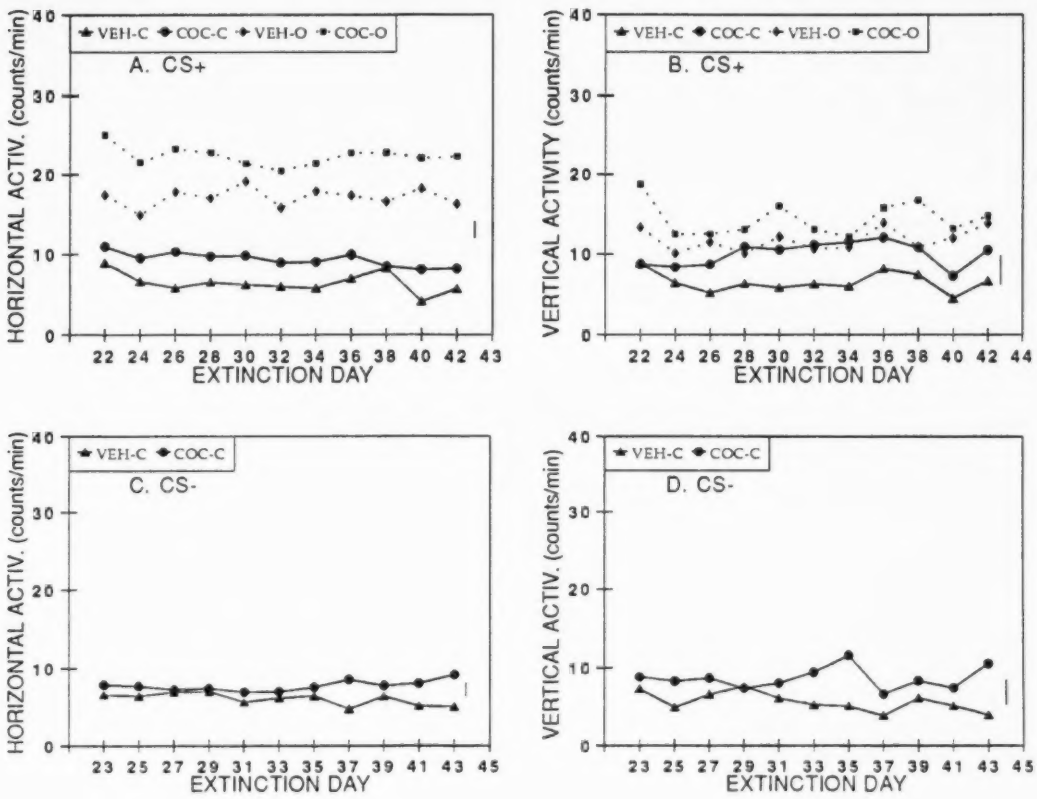

Figure 21.

(A) Mean horizontal activity (counts/min) in the cocaine-associated compartment (CS+) during extinction days in rats habituated to test boxes prior to conditioning, in cocaine (COC, 10 mg/kg, IP)- or vehicle (VEH)-trained groups conditioned with the tunnel closed (C) or with the tunnel open (O). Both COC-O and VEH-O groups were more active than the COC-C and VEH-C group, respectively, and horizontal activity was sustained in both COC groups at rates higher than in VEH groups throughout the extinction period. The critical difference at $p < 0.05$ is 2.13, multiple F-test (bar in lower right corner). Legends continued on following page.

(B) Mean vertical activity (counts/min) in the CS+ compartment during 8 extinction days in rats not habituated to test boxes prior to conditioning. Both COC-O and VEH-O groups were more active than the COC-C and VEH-C groups, respectively. The VEH-O group became significantly more active than the COC-O group over days, but there were no significant differences between COC-C and VEH-C. The C.D. at $p < 0.05$ is 2.77, multiple F-test (bar in lower right corner).

(C) Mean horizontal activity (counts/min) in the vehicle-associated compartment (CS-) during extinction days in rats not habituated to test boxes prior to conditioning, comparing the horizontal activity in COC and VEH groups conditioned with the tunnel closed (C). There were no significant differences between groups. The C.D. at $p < 0.05$ is 1.42, multiple F-test (bar in lower right corner).

(D) Mean vertical activity (counts/min) in the CS- compartment during extinction days in rats not habituated to test boxes prior to conditioning, comparing the vertical activity in COC and VEH groups conditioned with the tunnel closed (C). There were no significant differences between groups, but vertical activity did decrease in the COC-C group over days. The critical difference at $p < 0.05$ is 2.58, multiple F-test (bar in lower right corner) [n=12].

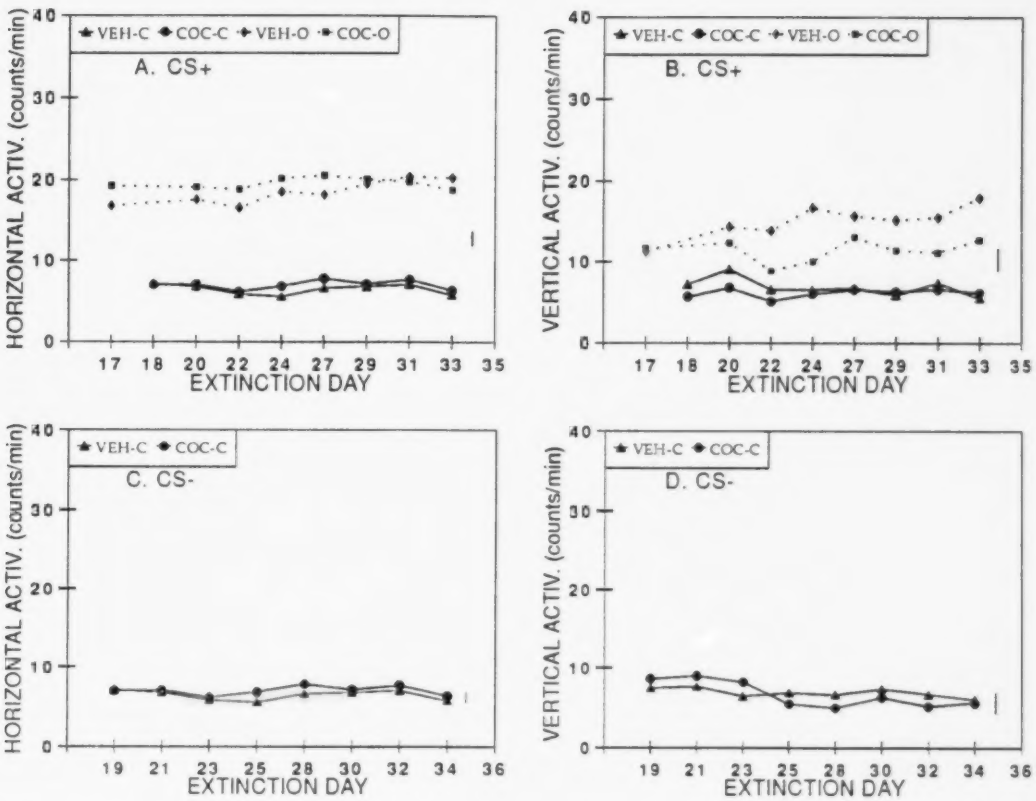

Figure 22. (A) Mean horizontal activity (counts/min) in the CS+ compartment during extinction days in rats not habituated to test boxes prior to conditioning, in cocaine (COC, 10 mg/kg, IP)- or vehicle (VEH)-trained groups trained with tunnel closed (C) or tunnel open (O). Both COC-O and VEH-O groups were more active than the COC-C and VEH-C groups, respectively, but there were no significant differences between COC-O and VEH-O, or between COC-C and VEH-C. The critical difference at $p < 0.05$ is 1.82, multiple F-test (bar in lower right corner).

5.3.8. EXTINCTION PERIOD (VERTICAL ACTIVITY)

Vertical activity for habituated groups during extinction is shown in Figures 21B (CS+) and 21D (CS-). Previously cocaine-conditioned groups displayed significantly more vertical activity than vehicle-conditioned groups in the CS+ compartment, and this was especially obvious in groups conditioned with the tunnel closed. Tunnel open groups were more active than tunnel closed groups, but the differences between tunnel open groups decreased over days, while that between tunnel closed groups increased over days. ANOVA revealed main effects of tunnel ($F(1,42)=12.18$, $p=0.001$), drug ($F(1,42)=5.1$, $p=0.029$), and days ($F(10,420)=3.6$, $p<0.001$). On vehicle extinction days, previously cocaine-conditioned rats were more active than vehicle-conditioned rats in the CS- compartment. There was a main effect of drug ($F(1,20)=4.77$, $p=0.041$).

The extinction results for vertical activity in non-habituated groups is displayed in Figures 22B (CS+) and 22D (CS-). In the CS+ compartment, activity increased over days in the tunnel open group previously conditioned with vehicle. There was an tunnel x drug x days interaction ($F(7,308)=2.04$, $p=0.05$). In the CS- compartment, vertical activity decreased over days, indicating a main effect of days ($F(7,154)=2.48$, $p=0.019$), but there was no significant effect of drug.

5.4. DISCUSSION

The primary purpose of the present experiments was to examine possible reasons for the apparent lack of conditioned horizontal and vertical motor activity previously observed in a cocaine-CPP procedure. Increases in horizontal and vertical activity have

often been reported when untreated rats were placed in a single-box environment previously associated with psychomotor stimulant (including cocaine) treatment (Beninger and Hahn, 1983; Beninger and Herz, 1986; Martin-Iverson and McManus, 1990; Stewart and Vezina, 1991; CHAPTER 2; CHAPTER 3). These increases are usually interpreted as being a function of the classical conditioning of drug effects, although this interpretation has been questioned (Gold et al., 1988; Damianopoulos and Carey, 1992; Willner et al., 1992; Szechtman et al., 1993; Martin-Iverson and Fawcett, in press). However, no such increases in motor activity were detected in a recent cocaine-CPP experiment, although place preferences were conditioned by cocaine (chapter 4). Indeed, motor activity measures were higher in the CS- compartment (paired explicitly with vehicle treatments) than in the CS+ compartment (paired with cocaine treatments).

These findings were replicated in the present experiment with n=24 for each group. CPP occurred with cocaine, and were extinguished. Horizontal and vertical activity rates/min were found to be higher in the CS- than the CS+ compartment, when calculated across the entire time spent in each compartment. However, motor activity over the period of testing in these experiments is a decreasing function of duration of testing. Therefore, the longer a rat is in a compartment, the lower it's average rate of activity will be. When only the first 5 minutes of time spent in each compartment (to equate the compartments for test duration) were analysed, no differences in horizontal or vertical activity between the CS+ and CS- compartments were observed (Figure 19A). Thus, the observed increase in CS- motor activity is most likely a function of test duration, which itself is dependent on CPP. When test duration is accounted for, there is no difference in

horizontal and vertical activity between CS+ and CS- compartments, contrary to the increase in activity in the CS+ compartment expected on the basis of classical conditioning.

A number of characteristics of the horizontal and vertical activity measures other than lack of difference in activity rates between the CS+ and CS- compartments are also incompatible with a classical conditioning interpretation. Firstly, rats that had cocaine paired with one (or both) compartments did not exhibit conditioned increases in vertical activity, even though vertical activity sometimes provides more robust classical conditioning effects than horizontal activity in single-box tests (Beninger and Hahn, 1983; Beninger and Herz, 1986). Secondly, the post-training increase in horizontal activity in the cocaine groups habituated to the test boxes before training did not extinguish. Thirdly, there was no evidence of classical conditioning of horizontal activity in the rats conditioned to cocaine in the open-tunnel condition if they were not habituated to the boxes prior to training. Finally, differences in vertical activity between habituated cocaine- and vehicle-treated groups occurred only during extinction, and had a tendency to increase over days.

There are two major differences between our CPP procedure and typical single-box conditioned activity procedures which may have contributed to the differences in results from these procedures. 1) In the CPP procedure, the conditioning occurs in a two compartment box, with the rats confined to one compartment during drug treatments, but testing for CPP occurs with free access to both compartments through a tunnel. The activity conditioning procedure is done in a single compartment box which is not changed

from the conditioning trials on the test day; 2) In our CPP procedure, rats are habituated to both compartments prior to conditioning, whereas this is not commonly done in the activity conditioning procedure. According to learning theory (Mackintosh, 1974), the presence of an habituation period should lead to the development of latent inhibition, preventing or reducing the conditioning of motor activity. The present experiments tested the effects of both of these differences in a procedure to determine if they are responsible for the absence of conditioned motor activity in the CPP procedure.

Testing rats for conditioning with access to both compartments after training with confinement to one compartment may account for the lack of conditioned motor effects. It is possible that opening the tunnel altered the environment such that the testing context was different enough from the training context that generalization was limited. Alternatively, opening a previously closed tunnel may be a more salient stimulus than conditioned cues, and behaviour induced by presence of the tunnel or thigmotaxic behaviour directed towards the tunnel might mask any conditioned motor effects. These two possibilities were tested by training and testing separate groups of rats with the tunnel always left open. In rats that are both trained and tested in the two-compartment box with the tunnel always open, horizontal activity was higher on a drug-free test in habituated rats conditioned with cocaine, relative to vehicle controls, but not in non-habituated rats (Figure 20A). This difference in habituated groups was maintained following a similar test after extinction (Figure 20B). Since habituated rats that previously had cocaine paired with an always-open tunnel increased horizontal activity on a drug-free test day, it initially appears that the effects of the presence of a tunnel *per se*, can be excluded as a reason for

the lack of motor conditioning in the CPP procedure. However, since rats in CHAPTERs 2 and 3 were also not habituated to the test boxes before conditioning but did display conditioned locomotion, it appears that the tunnel does affect motor conditioning. Taken together, these results suggest an interaction between the effect of the tunnel and the effect of habituation. However, there were also differences in absolute rates of horizontal activity in the groups trained and tested with tunnels always open and those trained with the tunnels closed but tested with the tunnels open. On the drug free test day with the tunnel open, rats conditioned with the tunnel open exhibited lower rates of horizontal activity than rats conditioned with the tunnel closed. Activity rates in the cocaine-treated, tunnel-open group were on par with those in the vehicle-treated, tunnel-closed group, and those in the vehicle-treated, tunnel-open group were significantly lower than the rates in the vehicle-treated, tunnel-closed group. This is despite the observation that rats in the open tunnel condition exhibited higher activity levels during conditioning. Thus, rats with similar experience with contextual cues differ in their rates of activity on a test day with equivalent conditions (tunnel open in all groups) depending on whether this prior experience was with tunnels open or closed. The opening of a tunnel that was closed during conditioning may therefore be partly responsible for the increase in horizontal activity in the CS+ and CS- compartments in rats trained with the tunnel closed. Furthermore, that rats conditioned only with vehicle in the tunnel closed condition exhibited higher levels of motor activity when the tunnel was opened than during training, or than rats conditioned with vehicle with the tunnel open on the same test day, indicates that stimulus change (i.e. opening a previously closed tunnel) can activate motor activity

in rats. This is relevant because it has previously been argued that "conditioned" motor activity in single compartment procedures is at least partly due to changing stimulus conditions on a non-drug test day by removing drug cues (Martin-Iverson and Fawcett, in press). That a relatively small change in stimulus conditions (opening a previously closed tunnel) can activate motor activity supports this contention. However, this "opening of the tunnel effect" cannot account for the differential effects of vehicle and cocaine.

The possibility that habituation to the test boxes before conditioning may have led to latent inhibition that blocked conditioned motor activity was also tested. However, there were no differences in horizontal and vertical behaviour on the non-drug test day between closed tunnel conditioned groups that were habituated to the test environment prior to conditioning and those who were not habituated (Figure 19A). There was an effect of habituation on conditioned motor activity in groups conditioned with the tunnel open, but rather than producing latent inhibition that might attenuate classical conditioning, an apparent conditioned effect on horizontal activity was only observed in groups with habituation prior to conditioning with the tunnel open (Figure 20A). In addition, the rate of sensitization of horizontal activity to cocaine during the conditioning period in cocaine groups conditioned with the tunnel open or closed (Figure 18A) was greater in habituated rats, in comparison to non-habituated cocaine groups (Figure 18B). Therefore, the failure to observe selective increases in CS+ motor activity is not due to the development of latent inhibition from pre-exposure to the test boxes.

The fact that there was no conditioned vertical activity in cocaine groups is contrary to reports from single-compartment experiments with cocaine (Beninger and

Herz, 1986) and amphetamine (Beninger and Hahn, 1983; Vezina and Stewart, 1991). However, since cocaine did not increase vertical activity in any of the groups during training, and indeed tended to decrease vertical activity (Figures 18C and 18D), the absence of conditioned vertical activity is not surprising. Stimulant-induced rearing appears to depend on structural features of the test boxes, such as size (Sullivan et al., 1992), and the present lack of cocaine-induced or conditioned vertical activity may be due to the type of boxes used.

The two factors of habituation and tunnel seemed to contribute greatly to the development of sensitization to horizontal activity during training (Figures 18A and 18B) in ways not comprehensible within a classical conditioning framework. Rats developed sensitization to cocaine more quickly in the open tunnel groups if they were habituated to the test box before training, while only habituated rats in the closed-tunnel condition developed sensitization; sensitization was not apparent in the non-habituated rats in the closed-tunnel condition. Activity rates were much higher in all groups in the open-tunnel condition than in the closed-tunnel condition throughout training. Thus, the highest levels of horizontal activity were observed in the cocaine group in the habituated open-tunnel conditions (Figure 18A).

Another line of evidence against classical conditioning as an adequate interpretation for the obtained behavioural effects, is that such effects did not always extinguish with repeated exposure to the contextual cues in the absence of drug treatment. During the extinction period in the closed tunnel groups, habituation (Figures 21A, 21B, 21C and 21D) but not absence of habituation (Figures 22A, 22B, 22C and 22D) resulted in greater

horizontal and vertical activity in previously cocaine-conditioned rats in comparison to respective vehicle rats in both compartments. Similar to the closed-tunnel group, habituation (Figures 21A and 21B) but not absence of habituation (Figures 22A and 22B) in open-tunnel groups, resulted in more horizontal and vertical activity in previously cocaine-conditioned rats. On the extinction test day, there was no effect of habituation in the first 5 minutes of the test in the CS+ and in the CS- in groups conditioned with a closed tunnel, although horizontal activity did not extinguish in the CS- compartment (Figure 19B). In the habituated cocaine group conditioned with the tunnel open, extinction did not occur (Figure 21A). Thus, habituation resulted in a stronger cocaine effect than no habituation on horizontal activity on the conditioning test, but this effect was sustained throughout the extinction period. Since horizontal activity did not extinguish, it does not fit the typical case for a classically conditioned response.

The appearance of sensitization of horizontal activity to cocaine as assessed by challenge injections after extinction has been taken by Stewart and Vezina (1991) to indicate the presence of non-associative sensitization to amphetamine. A similar effect could not be replicated by Ahmed et al. (1993). However, treatment regimen may account for the differences since Ahmed et al. (1993) did not allow an intervening day between conditioning blocks, as was the case with Stewart and Vezina (1991). Previous results have shown that intermittent administration of psychomotor stimulants with a spacing of 2 or 3 days between injections results in the most robust sensitization (Post, 1981; Robinson and Becker, 1986; Robinson and Berridge, 1993). Indeed, our present results indicate much more robust sensitization to cocaine when given every second day than our previous

single compartment results with daily cocaine treatments (Burger and Martin-Iverson, 1994; CHAPTER 2; CHAPTER3). Hence, the intervening days between injections in the Stewart and Vezina (1991) study may have produced stronger sensitization. In the present case, both results pertain. Sensitization to cocaine was observed in both CS+ and CS- compartments after extinction in the habituated closed-tunnel trained group when challenged with cocaine with the tunnels open and with the tunnels closed (Figures 19C and 19D). However, rats that were not habituated did not exhibit sensitization after extinction with the tunnels open, although sensitization to horizontal activity was observed in the CS- compartment when the tunnel was closed. Furthermore, neither the habituated nor the non-habituated groups trained with the tunnel open exhibited sensitization upon a challenge dose of cocaine when the tunnels were open (Figure 20C). Only the habituated group trained with the tunnel open exhibited sensitization and only when the tunnel was closed (Figure 20D). Thus, the appearance of post-extinction sensitization appears to depend on 1) prior habituation to the test apparatus, 2) whether the rats were trained with the tunnels open or closed, and 3) whether the rats are tested with the tunnels open or closed. If post-extinction sensitization is due to non-associative factors then none of these three environmental/experiential factors should have affected the expression of sensitization. That the environment and previous experience with the environment does affect post-extinction sensitization suggests it does not provide a measure of non-associative sensitization. In addition, a simple two-component model of sensitization consisting of an associative and a non-associative component cannot explain the present results. A three component model has been proposed by Szechtman et al. (1993),

consisting of associative, non-associative, and behavioural components to describe sensitization to quinpirole. However, while such a model may explain the differential effects of training with the tunnel open or closed, it cannot account for the added effects of prior habituation.

The pattern of results observed in the present experiments do not appear to be amenable to interpretation by extant hypotheses, such as the classical conditioning hypothesis or the operant conditioning hypothesis of Willner et al. (1992). The operant hypothesis cannot account for the increase in horizontal activity in the CS- compartment, for the effects of habituation in the tunnel-open training condition, nor for the failure of extinction to extinguish the response under certain conditions. However, future experiments with this procedure should include home cage groups to better control for context-specificity, and also randomized controls who receive cocaine injections and confinement in both compartments to better control for the effects of the testing environment. Although these hypotheses cannot explain the increase in horizontal activity, it is still fair to say that horizontal activity, as measured by photobeam interruptions, was greater among cocaine groups than among vehicle groups. However, work by Bardo et al. (1984) has shown that during the extinction of morphine-CPP in a three compartment procedure, although the duration per entry into the drug-associated compartment extinguishes, the number of entries into the drug-ssociated compartment increases. Therefore, if this effect is also present with extinction of cocaine-CPP, then it is possible that it may have confounded the expression of some type of classically conditioned motor activity. On the other hand, it is doubtful that the photobeam array in our test boxes can

accurately describe an ongoing behaviour, since this array is more effective at calculating the frequency than the duration of a behaviour. For example, a rearing rat may interrupt a photobeam, but if it does not move from that spot, then only one "rear" may be counted, although the animal may spend a considerable total duration of time in that posture. It has been argued (Martin-Iverson and Fawcett, in press) that locomotion and rearing (horizontal and vertical activity) are inappropriate behaviours to measure in single-box classical conditioning procedures. Based on video records of rats' behaviours during conditioning with amphetamine or PHNO it was suggested that increases in these behaviours on a drug-free test-day are unlikely to be due to classical conditioning. The present results indicate that structural features of the testing environment, such as the presence or absence of a tunnel, and the presence or absence of prior habituation to the testing environment have strong influences on both so-called "classical conditioning" and sensitization to cocaine. It appears that measures of horizontal and vertical activity are determined by so many complex factors that they may not provide a particularly robust measure of either classical conditioning of stimulant responses or sensitization. A similar point has been made by Damianopoulos and Carey (1992, 1993) with regard to sensitization to cocaine and apomorphine. They have termed the complex interplay among stimulus features of the environment, behaviour, learning and drug effects, "behavioural reorganization" in determining changes in response to drugs with repeated treatments. Similarly, the present results appear to support such a complex concept in the sensitization and in the conditioning of stimulant-induced behaviours. Our results suggest that only a comprehensive analysis of behaviour by direct observation may be sufficient to accurately

describe behaviour. Perhaps determining why the presence of a tunnel and habituation to the testing environment can alter the effects of repeated treatments of cocaine may lead to a more adequate understanding of the behavioural effects of stimulants than is possible with current hypotheses.

CHAPTER 6. GENERAL DISCUSSION

6.1. DISCUSSION

This thesis had several objectives. These included 1) determining the role of dopamine and L-type Ca^{2+} channels in i) the establishment and the expression of cocaine-conditioned motor effects, ii) in the conditioned reinforcing effects of cocaine, and in iii) cocaine-induced behavioural sensitization; and 2) determining if the effect of cocaine on locomotion (horizontal activity) and rearing (vertical activity), in a two-compartment testing apparatus, could be adequately explained by classical conditioning.

The results in CHAPTER 2 support previous results in single-compartment procedures with intermittent administration regimens (Barr et al., 1983; Beninger and Herz, 1986) which indicated that environment-specific cocaine-induced (10-20 mg/kg, IP) behavioural sensitization of locomotor activity occurs. Following a cocaine challenge injection (10 mg/kg, IP) in experiment 1 of CHAPTER 2, sensitization was not present in the test boxes in rats that received cocaine injections paired with a different context (home cage environment). Cocaine-conditioned locomotion was also established in the test context, and only groups displaying a conditioned locomotor effect to cocaine exhibited behavioural sensitization to the challenge injection of cocaine. Sensitization to cocaine (Post et al., 1981; Weiss et al., 1989) and amphetamine (Tilson and Rech, 1973; Stewart and Vezina, 1991) has previously been found to be context-specific. However, in the results of CHAPTER 2, there appeared to be a pharmacological dissociation between sensitization and classical conditioning, since the D_2 receptor antagonist haloperidol

attenuated the development of sensitization during the conditioning period, but had no effect on establishment of classical conditioning. The dihydropyridine nimodipine also attenuated the development of sensitization during conditioning, but completely blocked establishment of cocaine-conditioned locomotion. This suggests that blockade of the establishment of cocaine-conditioned locomotion by pimozide (Beninger and Herz, 1986) was due to effects of pimozide on L-type Ca^{2+} channels. These results show that sensitization and conditioning to cocaine may be pharmacologically dissociated. However, in future studies, to determine if nimodipine and/or haloperidol do block the establishment of cocaine-conditioned locomotion, a cocaine challenge injection must be administered to all groups in the absence of nimodipine and/or haloperidol.

In CHAPTER 3, also in a single-compartment procedure, it was observed that the expression of cocaine-induced sensitization of locomotor activity was blocked by nimodipine, although blockade of the expression of cocaine-conditioned locomotion required both nimodipine and haloperidol. These results indicate that the expression of sensitization and classical conditioning of locomotor effects induced by cocaine may be pharmacologically dissociated, as was reported for the establishment of these effects, and therefore support the theory that different processes may underlie sensitization and classical conditioning.

It was previously reported that the expression of cocaine-conditioned locomotion was not blocked by pimozide (Beninger and Herz, 1986), although pimozide is an equipotent antagonist of L-type Ca^{2+} channels and D_2 dopamine receptors. However it is possible that the dose of pimozide used provided a different level of blockade of L-type

Ca^{2+} channels and D_2 receptors than that which occurred with the doses of haloperidol and nimodipine that were used in CHAPTER 3.

The pattern of results observed in CHAPTER 3 resembles those of others (Gold et al., 1988; Damianopoulos and Carey, 1992), where sensitization during the conditioning period is only apparent when considered as a percent of control, and did not increase in absolute number of activity counts over the 10 conditioning days. When continued over 14 days (Burger and Martin-Iverson, 1994), cocaine (10 mg/kg, IP) did produce sensitization of locomotor counts, as did PHNO over a 10 day period (Martin-Iverson and McManus, 1990). However, after intervening non-drug days, groups with prior cocaine treatments did exhibit sensitization, indicating that repeated treatments with cocaine need intervening non-drug days for sensitization to become apparent. Others (Post, 1981; Robinson and Becker, 1986; Robinson and Berridge, 1993) have also reported that sensitization is more clearly apparent when treatments are on alternate days.

The results in CHAPTER 4 indicated that the establishment of the conditioned reinforcing properties of cocaine, as measured by CPP, depends on the activation of L-type Ca^{2+} channels. These results support those with another dihydropyridine L-type Ca^{2+} channel antagonist isradipine (Pani et al., 1991) where cocaine-CPP was blocked at 3 non-sedating doses, and are similar to those found with nimodipine (10 mg/kg, SC) for the establishment of cocaine-conditioned locomotion in experiment 1. However, CPP may be a more sensitive measure of cocaine-induced effects than locomotor activation since nimodipine blocked CPP at 3 non-sedating doses (0.1, 1.0, and 10.0 mg/kg, SC), and only the 10 mg/kg dose reduced cocaine-induced motor activity during conditioning in the CPP

procedure. When a challenge injection of cocaine (10 mg/kg, IP) was administered on the day following the drug-free CPP test, CPP was only blocked in rats previously treated with 1.0 mg/kg of nimodipine.

The most effective dose of nimodipine appeared to be the 1.0 mg/kg dose, and the 3 doses used formed an inverted U dose-response in the blockade of CPP after a challenge injection of cocaine. Inverted U-shaped dose-response curves have been observed before with nimodipine in a number of behavioural tasks (Cunningham et al., 1990; Thompson et al., 1990; LeVere et al., 1990), although a satisfactory explanation of the mechanisms involved has not been formulated.

The cellular location of L-type Ca^{2+} channels is not clear. Although dihydropyridine L-type Ca^{2+} channel antagonists reduce cocaine-induced dopamine overflow (Pani et al., 1990a,b) and occupation of dopamine receptors (Burger and Martin-Iverson, 1994), indicating that these Ca^{2+} channels are presynaptic, there is also evidence that they can be postsynaptic. For example, in bass retinal horizontal cells, activation of L-type Ca^{2+} currents by dopamine can be blocked by the D_1 receptor antagonist SCH23390 (Pfeiffer-Lin and Lasater, 1993).

In the results of CHAPTER 4, an unexpected result occurred on the drug-free test day for CPP. In cocaine-conditioned rats, the greatest levels of horizontal and vertical activity occurred in the CS- compartments, where the rats had never received cocaine during conditioning. In the experiments performed in CHAPTER 5, these results were replicated, but when the data were examined more closely it was determined that the observed increase in activity in the CS- compartment was a function of sampling duration,

which is dependent on CPP. When test duration is accounted for, or when CPP is extinguished, there were no differences in horizontal or vertical activity between CS+ and CS- compartments. Of course, these findings are still contrary to what is expected on the basis of classical conditioning, where an increase in activity was expected in the CS+ compartment.

There were several other reasons that a classical conditioning interpretation for horizontal or vertical activity did not appear to be feasible. 1) The appearance of "conditioned" horizontal activity depended on whether habituation was present in groups conditioned with the tunnel open but not in those conditioned with the tunnel closed. 2) Extinction only occurred in non-habituated rats conditioned with the tunnel closed. 3) Conditioned vertical activity was absent, although vertical activity sometimes provides a more robust measure of classical conditioning than does horizontal activity (Beninger and Hahn, 1983; Beninger and Herz, 1986). However, structural features of the test environment appear to affect stimulant-induced rearing (Sullivan et al., 1992), and there were no significant differences in vertical activity between respective cocaine and vehicle groups during conditioning.

The possibility of latent inhibition was also tested. However, on the drug-free test day, there were no differences between habituated and non-habituated cocaine groups conditioned with the tunnels closed. On the other hand, on the drug-free test in groups conditioned with the tunnels open, only the habituated cocaine group displayed a significant increase in horizontal activity. In addition, the rate of sensitization of horizontal activity was much greater in habituated than non-habituated cocaine groups during the

conditioning period. Furthermore, prior habituation resulted in a stronger effect on horizontal and vertical activity during extinction than no prior habituation. These results appear to effectively rule out latent inhibition, since if anything, latent inhibition should have reduced a conditioned effect on horizontal activity in habituated rats.

In the results of CHAPTER 5, it was observed that post extinction sensitization depends on 1) prior habituation to the test apparatus, 2) whether the rats were trained with the tunnels opened or closed, and 3) whether rats are tested with the tunnels opened or closed. If post extinction sensitization is due to non-associative factors, then environmental/experiential factors should not have affected the expression of sensitization. Therefore, post extinction sensitization may not provide a measure of non-associative sensitization.

The pattern of results observed in CHAPTER 5 do not appear to be explicable by interpretation through hypotheses such as classical or operant conditioning (Willner et al., 1992); the three-component model of Szechtman et al. (1993), consisting of associative, non-associative, and behavioural components; by disconfirmation of an expectancy; or by generalization (Mackintosh, 1974).

The operant hypothesis would not predict an increase in activity in the CS-compartment or an absence of an effect of extinction. In addition, operant conditioning cannot account for an effect of habituation that depends on whether the tunnel was opened or closed. For example, during conditioning, activity was greater in habituated than non-habituated cocaine groups conditioned with the tunnel open or closed. However, regardless of previous experience with the environment, activity was not significantly different on the

drug-free test in the tunnel closed groups, nor was it significantly different on the cocaine challenge test in groups conditioned with the tunnel open. On the other hand, operant conditioning could account for the increase in activity on the drug- free test between groups conditioned with the tunnel open, since these groups differed in activity during conditioning. Similarly, operant conditioning could account for the difference in activity between tunnel closed conditioned groups on the cocaine challenge test day. Therefore, not all effects on horizontal and vertical activity can be explained by operant conditioning.

The three-component model (Szechtman et al., 1993) also explains some effects of CHAPTER 5, but not others. For example, horizontal activity was context-dependent in the post-extinction sensitization test in the non-habituated closed tunnel group, since it was absent following extinction in the tunnel open test. The three component model also predicts a behavioural-dependent component that relies on structural features of the test environment. In our results, this effect may have been observed on the drug-free test, since equal activity rates occurred in both the CS+ and the CS- compartments. However, a better example of a behaviour-dependent component in our results was apparent in open tunnel groups, since habituated groups were more active than non-habituated groups both during conditioning and on the drug-free test. The three component model also predicts a context-independent component, and this was observed in our results on the post extinction cocaine challenge test, since sensitization appeared in groups that had been extinguished. However, the three-component model cannot account for the effect of prior experience with the environment. For example, post extinction sensitization was dependent on habituation versus no habituation, and similarly, horizontal activity was "conditioned" in both closed tunnel groups but only in habituated open tunnel groups.

During conditioning with psychomotor stimulants, an animal develops certain expectancies. These include subjective/cognitive effects such as an increase in heart rate and an increase in motor activity. There is also an affective component which includes effects such as the euphoria or "feeling good". Therefore, disconfirmation of the expectancy of drug-related cues in the CS+ compartment on the drug-free test day may result in frustrative non-reward, which can be behaviourally activating (Mackintosh, 1974), or result in increased exploratory activity simply due to stimulus change. However, these effects cannot account for the increase in activity in the CS- compartment, since cocaine was never administered in the CS-.

Finally, generalization does not explain the increase in activity in the CS- compartment on the drug-free test day in rats conditioned with the tunnel closed, since although there were some structural differences between the two compartments (floor type), there were no significant differences in activity between compartments.

The results from CHAPTER 5 indicate that the structural features of the testing environment such as presence or absence of a tunnel, and experiential factors such as presence or absence of habituation can influence both so-called "classical conditioning" and behavioural sensitization. With current technology, it does not appear that measuring photobeam interruptions can provide an adequate description of behaviour. Therefore, it is possible that only an intensive visual examination of the complex behavioural effects induced by a drug-environment interaction may be necessary to understand the effects of stimulant drugs. For example, based on examination of videotapes of rat behaviours, it has been argued that neither horizontal nor vertical motor activity can be classically

conditioned, but that snout contact and sniffing may be (Martin-Iverson and Fawcett, in press).

6.2. CONCLUSIONS

The putative classical conditioning and sensitization of horizontal and vertical activity appear to be functions of context, prior experience, and behaviour in ways that may not be comprehensible in terms of classical conditioning or simple neural substrates (i.e., receptor changes, increased dopamine release, etc.). Although a detailed behavioural/environmental/learning approach requiring intensive visual examination of rat behaviours would be time consuming and more expensive, it appears that it may be necessary to understand what is happening.

Nimodipine may not block classical conditioning, but rather some other as yet undefined complex learned behavioural effect to cocaine, i.e., a change in behaviour as a function of an interaction between experience, environment, and drug effects. On the other hand nimodipine may just reduce excitability of neurons such that drug effects are absent from the picture.

It is clear that previous experience with an environment, the type of environment, and repeated experience with a drug in a particular environment can alter the behaviours of animals in a specific context both in the presence, and in the absence of cocaine. Some of these effects can be blocked with L-type Ca^{2+} channel antagonists, which suggests that the excitability of neurons, probably via dopamine neurotransmission, is important in the mediation of these effects. Whether the development of cognitive expectancies or more

subtle interactions between or among environment-ongoing behaviour and drug effects are more important, is not presently clear. Further work is necessary to determine which processes are responsible and to determine which processes L-type Ca^{2+} channels are involved in.

Although the results of CHAPTER 5 could not be explained by classical conditioning, future studies with this experimental design should include home cage controls to better control for context-dependency. Another control group that would strengthen the results would be one that received cocaine injections in both compartments, thus providing a more effective control for the effects of floor type.

Other experimental designs may have also helped to understand these results. For example, a combination of CPP and self-administration might overcome any negative associations that the animals develop to the injection and injection procedure. We attempted to develop such a procedure where the rats had access to a cocaine solution in a 10 ml syringe that had been fitted with a drinking nipple. Unfortunately, the rats would not drink the cocaine solution.

Another possibility to control for the effect of floor types, would be to have boxes that have either grates or bars on both floors. Following conditioning in such a box, the rats could be tested for conditioning in a box with the opposite floor type, or in a box with grates on the floor in one compartment and bars in the other, or be restricted to one or the other floor type. However, this may be too expensive, but could possibly be accomplished with boxes that had removeable floors.

It is also possible that the tunnel that separated the two compartments may activate species specific behaviours such as burrowing or thigmotaxis. Other test boxes could possibly include a tunnel that is adjustable for length and height. The inclusion of an habituation period before conditioning in a single compartment procedure could also help to determine more about the effect of the tunnel, and about the results observed in the open tunnel cocaine groups.

6.3. CLINICAL IMPLICATIONS

There is increasing support from animal studies suggesting a role for dihydropyridine L-type Ca^{2+} channels antagonists in the clinical treatment of stimulant abuse and stimulant-induced psychoses. Nimodipine and isradipine block cocaine-induced dopamine overflow and motor activity in rats (Pani et al., 1990a, b). Nifedipine blocked amphetamine-induced motor activation in mice (Grebb, 1986), and nitrendipine blocked cardiac toxicity and lethal effects induced by cocaine (Trouve and Nahas, 1986).

Classical conditioning of psychomotor stimulant effects has been implicated in stimulant addiction and in the development of behavioural sensitization. Former cocaine addicts exhibit strong cravings and drug-like physiological responses when presented with drug-associated paraphernalia (O'Brien et al., 1988; Muntaner et al., 1990). It has been suggested that behavioural sensitization to psychomotor stimulants provides a model for stimulant-induced psychoses, and possibly for schizophrenic psychoses (Angrist, 1983; Robinson and Becker, 1986). Work from this thesis and other research (Spyraki et al., 1982b; DiChiara and Imperato, 1987; Pani et al., 1991; DiLullo and Martin-Iverson,

1992a,b; Robinson and Berridge, 1993) suggests that dopamine plays an important role in reinforcement and in stimulant-induced effects. CPP is thought to provide a model for the conditioned reinforcing effects of drugs (Swerdlow et al., 1989), and the establishment of cocaine-CPP has been blocked by isradipine (Pani et al., 1991a) and by nimodipine (CHAPTER 4). These CPP results suggest that the conditioned reinforcing effects of cocaine may be blocked by nimodipine alone. On the other hand, it is the expression of CPP that is important with a victim of drug abuse, since the conditioned reinforcing effects of the drug have already been established. A recent clinical study (Rosse et al., 1994) found that cue-induced cocaine craving was not decreased by nimodipine alone. Since nimodipine alone blocked the expression of behavioural sensitization induced by a cocaine challenge, it is possible that L-type dihydropyridine Ca^{2+} channel antagonists may effectively treat stimulant-induced psychoses. In addition, if the animal models used in CHAPTER 3 have construct validity, then the results from CHAPTER 3, and past results (DiLullo and Martin-Iverson, 1992b) suggest that the combination of a D_2 dopamine receptor antagonist and a dihydropyridine L-type Ca^{2+} channel antagonist could provide a treatment for psychomotor stimulant addiction.

Although the above results from CHAPTERS 2 and 3 and results from other single compartment conditioned locomotor activity studies in rats suggest possible pharmacotherapeutic treatments for stimulant-induced effects in humans, the results from CHAPTERS 4 and 5 suggest that the animal models used in the first two experiments may be too simplistic. Therefore, further study may be required before the results from CHAPTERS 2 and 3 can be confidently accepted.

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